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SEQUENTIAL USE OF PAIRED COMBINATIONS OF ISONIAZID, STREPTOMYCIN, PARA-AMINOSALICYLIC ACID, AND PYRAZINAMIDE[1, 2]

A UNITED STATES PUBLIC HEALTH SERVICE TUBERCULOSIS THERAPY TRIAL

(Received for publication August 12, 1959)

Introduction

Tuberculous patients still infectious after six months of continuous treatment are likely to remain so without more effective chemotherapy or supplementary procedures.

This conclusion emerges from the accumulated evidence of a series of clinical trials coordinated by the Public Health Service and conducted in cooperation with more than twenty-five state and municipal tuberculosis hospitals throughout the country. Since the first trial of isoniazid in 1952, uniform observations have been collected on more than 6,000 patients with pulmonary tuberculosis during their initial treatment with antimicrobial agents. In each trial and with each chemotherapeutic combination, comparable groups of patients have exhibited a typical bacteriologic response to treatment, illustrated in figure 1 by results from a group of patients treated with isoniazid—PAS. During the early weeks, the response to treatment is prompt and steady; at each successive period fewer patients continue to excrete tubercle bacilli. Infectiousness is reversed at a fairly rapid rate for the first sixteen to twenty weeks of treatment, then more slowly until virtually no further change occurs after twenty-four weeks.

Characteristics of the patients under treatment influence the rate and degree of bacteriologic response. The curve shown in figure 1 is for a population heterogeneous with respect to race, sex, age, stage of disease, and extent of cavitation. The effect of one of these factors is illustrated in figure 2, in which two somewhat more homogeneous groups have been achieved by dividing the population according to stage of disease. The difference between the curves reflects the less rapid and less frequent reversal of infectiousness

[1] Presented in part by Dr. Harold Curtis of Cleveland, Ohio, before the Medical Session, as part of *Section 1A*, at the annual meeting of the American Trudeau Society, Philadelphia, Pennsylvania, May 19, 1958.

[2] The trial was coordinated and the report prepared by Dr. Frank W. Mount and Shirley H. Ferebee of the Tuberculosis Program, United States Public Health Service, for the clinical investigators: Dr. R. C. Edson, Cedarcrest Sanatorium, Newington, Connecticut; Dr. G. C. Wilson, Uncas-on-Thames Sanatorium, Norwich, Connecticut; Drs. R. J. Davies, G. H. Hames, B. L. Brock, L. C. Manni, and A. G. Lewis, Florida State sanatoriums at Lantana, Orlando, Tallahasee, and Tampa, Florida; Dr. R. F. Corpe, Battey State Hospital, Rome, Georgia; Dr. L. H. Berard, Municipal Tuberculosis Sanitarium, Chicago, Illinois; Dr. E. G. Beacham, Baltimore City Hospital, Baltimore, Maryland; Dr. P. T. Chapman, Herman Kiefer Hospital, Detroit, Michigan; Dr. W. J. Steininger, William H. Maybury Sanatorium, Northville, Michigan; Dr. S. Cohen, Glen Lake Sanatorium, Oak Terrace, Minnesota; Dr. C. A. Brasher, Missouri State Sanatorium, Mount Vernon, Missouri; Drs. A. Goldman, B. Friedman, and M. B. Pianetto, Robert Koch Hospital, Koch, Missouri; Dr. R. B. Haas, USPHS Hospital, Brooklyn, New York; Dr. C. D. Thomas, North Carolina State Sanatorium, Black Mountain, North Carolina; Dr. H. I. Humphrey, Benjamin Franklin Sanatorium, Columbus, Ohio; Dr. R. H. Browning, Ohio State Sanatorium, Columbus, Ohio; Dr. H. G. Curtis, Sunny Acres Hospital, Cleveland, Ohio; Dr. G. M. Eckley, Henry R. Landis State Hospital, Philadelphia, Pennsylvania; Dr. George E. Martin, Pittsburgh Tuberculosis Sanatorium, Pittsburgh, Pennsylvania; Drs. E. P. Bowerman and F. H. Alley, Tennessee Tuberculosis sanatoriums at Memphis and Oakville, Tennessee; Dr. E. P. Roberts, San Antonio State Tuberculosis Hospital, San Antonio, Texas; and Dr. T. F. Sheehy, Firland Sanatorium, Seattle, Washington.

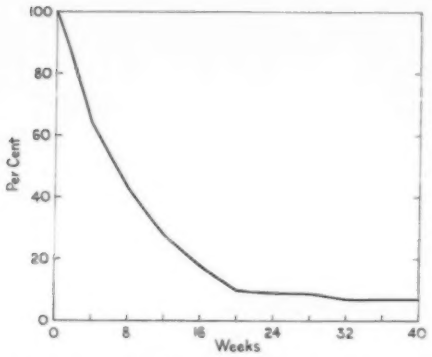

FIG. 1. Percentage of cultures positive for tubercle bacilli among patients treated with isoniazid—PAS.

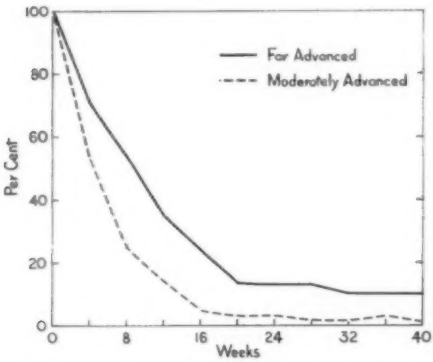

FIG. 2. Percentage of cultures positive for tubercle bacilli among patients with moderately advanced and far advanced disease treated with isoniazid—PAS.

with more extensive disease. Nevertheless, the curves have two common features, a rapid initial response and a plateau by at least the sixth month.

The type and dosage of chemotherapeutic agents also influence bacteriologic response, as shown in figure 3 for two heterogeneous groups, one treated with isoniazid—PAS and the other with biweekly streptomycin—PAS. The *difference* between the two curves demonstrates the superiority of isoniazid—PAS. Their *similarity* illustrates the *limits* of chemotherapy's influence on bacteriologic response. On *neither* regimen did any appreciable bacteriologic change take place after the first six months of treatment. The same pattern of response was observed with each of nine different continuous treatment regimens studied in this series of trials.

One of the trials was an investigation in 1953 to determine whether chemotherapy's effectiveness could be prolonged by changing the combination of drugs after the sixth month. Since only three drugs, isoniazid, streptomycin, and PAS, were at that time generally available, each patient was assigned to one of the following regimens:

First Course (0–24 Weeks)	Second Course (25–40 Weeks)
Isoniazid—PAS	Isoniazid—PAS
Isoniazid—PAS	Streptomycin—PAS
Streptomycin—PAS	Streptomycin—PAS
Streptomycin—PAS	Isoniazid—PAS

The bacteriologic results are shown in figure 4. Continued treatment with either combination of drugs produced the typical curve of reversal of infectiousness. Substitution of streptomycin for isoniazid after twenty-four weeks had no apparent effect, but a switch to isoniazid from streptomycin produced further bacteriologic change. These results suggest that, to be effective, the substituted combination must be superior, or at least equal, to the original combination.

The optimal effect of changing chemotherapy might be expected when the second course contained two, instead of one, previously unused antimicrobial agents. Since such an investigation required a fourth drug and a combination at

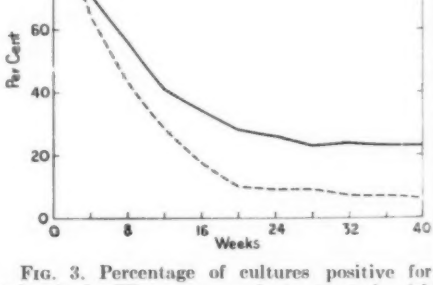

FIG. 3. Percentage of cultures positive for tubercle bacilli among patients treated with isoniazid—PAS and patients treated with biweekly streptomycin—PAS.

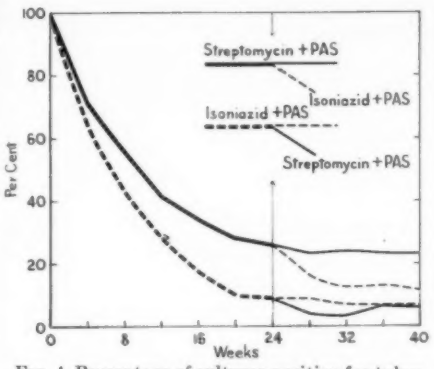

FIG. 4. Percentage of cultures positive for tubercle bacilli among patients treated with only one antimicrobial combination for forty weeks and among patients treated with one combination for twenty-four weeks followed by another combination for sixteen weeks.

least as effective as isoniazid—PAS, the use of pyrazinamide was considered.

Although patients treated with pyrazinamide in combination with either isoniazid (1, 2) or streptomycin (2) had shown considerable improvement, investigators were not agreed on its hepatic toxicity. At this point, the investigative group associated with the Public Health Service undertook a control study on the relation of pyrazinamide to liver damage (3). Toxic hepatitis occurred among 2 to 3 per cent of patients receiving a twelve-week course of daily doses of either 25 or 40 mg. of pyrazinamide per kg. in combination with isoniazid. The rate rose to 6.6 per cent when the 40 mg. per kg. dose was continued for twenty-four weeks. The relative infrequency of severe liver damage during the early weeks of treatment encouraged the investigative group to re-examine, with pyrazinamide as the fourth antimicrobial agent, the usefulness of changing regimens. The results observed are the subject of this report.

MATERIAL AND METHODS

Except on the control regimen, isoniazid—PAS for thirty-two weeks, all patients were given two sixteen-week courses of chemotherapy in which they received the four major drugs in paired combinations. These combinations and the sequence in which they were used are shown in table 1. Patients selected for the trial by the participating hospitals were randomly assigned in the central office to treatment regimens by procedures described in previous papers from the series (4).

The daily dose of isoniazid ranged from 4 to 6 mg. per kg. of body weight. Para-aminosalicylic acid was given in a daily dose of 10 to 12 gm. of acid PAS. Doses of other forms of PAS were adjusted to an equivalent amount of the acid. The daily dose of pyrazinamide ranged from 35 to 45 mg. per kg. of body weight. On all regimens using streptomycin, 1 gm. was given twice a week, except on the initial course on *Regimen 5*, when 1 gm. was given daily.

Admissions began in July, 1956, and by June, 1957, a total of 1,882 patients were under observation in twenty-five hospitals. The trial was limited to patients more than fifteen years old with pulmonary tuberculosis who had received no more than two weeks of previous chemotherapy. In addition to roentgenographic evidence of disease, bacteriologic proof of its etiology was required.

Clinical, bacteriologic, and roentgenographic examinations were made on each patient before his chemotherapy regimen was assigned, and at four-week intervals during treatment. Patients were given intradermal tests with intermediate strength PPD-S (5 TU) initially and after thirty-two weeks by physicians in the participating hospitals. Provision was made in the protocol for the responsible physician to change the assigned regimen for two reasons: severe worsening of a patient's condition which might be checked by a change in chemotherapy, and intolerance to one or both of the assigned drugs. As the trial was not primarily a test of pyrazinamide's hepatic toxicity, routine liver function studies were not required as research observations. However, nearly all hospitals tested liver function initially for later comparison, should signs or symptoms of hepatic disturbance appear, in order to protect patients from the risk of serious liver damage.

Initial characteristics of patients: Centrally controlled random assignment of treatment regimens resulted in five similar groups of patients. Selected characteristics of each group are presented in table 2.

TABLE 1

DRUG PAIRINGS AND SEQUENCE IN WHICH USED

Regimen	First Course (1–16 weeks)	Second Course (17–32 weeks)
1	Isoniazid—PAS	Isoniazid—PAS
2	Isoniazid—PAS	Streptomycin (biweekly)—pyrazinamide
3	Isoniazid—pyrazinamide	Streptomycin (biweekly)—PAS
4	Streptomycin (biweekly)—pyrazinamide	Isoniazid—PAS
5	Streptomycin (daily)—pyrazinamide	Isoniazid--PAS

TABLE 2

INITIAL CHARACTERISTICS OF 1,882 PATIENTS RANDOMLY ALLOCATED TO FIVE REGIMENS

Characteristics	Total	Regimens				
		1	2	3	4	5
Number of patients	1,882	384	368	380	369	381
	Per Cent					
Race and sex						
Male white	46.2	48.2	44.3	47.6	45.5	45.2
Male nonwhite	16.7	15.6	15.5	15.5	20.6	16.5
Female white	22.1	19.3	23.9	23.2	20.9	23.1
Female nonwhite	15.0	16.9	16.3	13.7	13.0	15.2
Age (in years)						
Less than 35	33.2	33.5	32.9	30.9	32.9	35.4
35–44	23.1	23.6	24.7	22.7	21.5	23.1
45–54	21.1	20.1	19.0	24.5	22.8	19.2
55 and older	22.6	22.8	23.4	21.9	22.8	22.3
Time since first hospital admission						
Less than 1 year	94.7	95.5	95.9	94.1	94.0	94.1
1 year or more	5.3	4.5	4.1	5.9	6.0	5.9
Stage of disease						
Minimal	10.7	12.5	8.4	11.3	11.4	10.0
Moderate	42.5	38.3	43.2	44.0	42.8	44.1
Far advanced	46.8	49.2	48.4	44.7	45.8	45.9
Cavitation						
None	36.8	36.5	35.1	37.6	38.8	36.2
Single	13.5	15.9	13.6	13.9	13.3	11.0
Multiple	49.7	47.6	51.3	48.5	47.9	52.8
Sputum culture						
Negative for tubercle bacilli	17.1	18.5	17.7	17.4	15.7	16.3
Positive for tubercle bacilli	82.9	81.5	82.3	82.6	84.3	83.7
Body weight						
Normal or overweight	38.1	39.2	38.8	37.6	38.7	36.0
Underweight*	61.9	60.8	61.2	62.4	61.3	64.0
Temperature						
Less than 99.0°F.	60.4	60.7	61.1	59.6	62.4	58.3
99.0°F. and over	39.6	39.3	38.9	40.4	37.6	41.7

* Minus deviation of more than 10 per cent from standard height-weight values.

Two-thirds of the patients were white, with twice as many men as women. The nonwhite patients were nearly equally divided by sex. Approximately one-third of the patients were less than thirty-five years old and those over that age were nearly equally divided among three age groups, thirty-five to forty-four, forty-five to fifty-four, and fifty-five years or older.

Ten per cent of the patients had only minimal disease, the remainder being made up of equal groups with moderately advanced or far advanced disease. No cavities were discernible on the initial roentgenograms of 37 per cent of the patients; 13 per cent had single cavities; and 50 per cent, two or more cavities.

Although bacteriologic evidence of disease was one of the criteria for eligibility, tubercle bacilli failed to grow on the initial sputum cultures of 17 per cent of the patients. Usually tubercle bacilli had been found on sputum smears or cultures prior to hospital admission but, when such examinations had not been made, patients could be entered in the trial on roentgenographic findings alone, pending subsequent laboratory studies completed at the hospital.

Sixty-two per cent of the patients were underweight and nearly 40 per cent were febrile on entrance to the trial. For most patients this was their first admission to a tuberculosis hospital, and the other 5 per cent had received no significant chemotherapy during their previous admission.

When a carefully designed scheme has been used to assign patients randomly to treatment regimens and when the resulting groups have similar initial compositions according to observed characteristics, the assumption is made that they are also alike with respect to other unobserved or unobservable characteristics. Occasionally, obser-

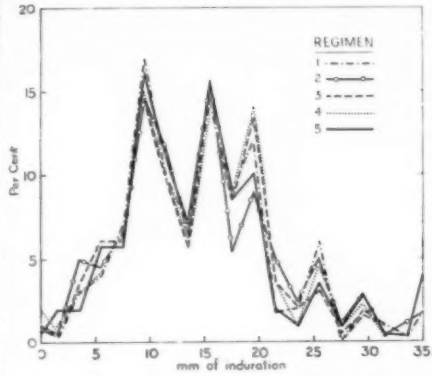

FIG. 5. Percentage distribution of sizes of initial tuberculin reactions among patients on 5 regimens. (High values on multiples of 5 reflect reader preference.)

vation of another characteristic provides an opportunity to check this assumption. In a previous trial, the results of pretreatment liver function studies were similar for each randomly determined treatment group (3). The present trial included initial tuberculin tests, using intermediate-strength PPD-S (5 TU) supplied by the central office. Test results are available for nearly all patients and the distribution of the transverse diameter of reactions is shown in figure 5 for the patients in each regimen. The similarity in results supports the assumption that these groups are alike in other respects. Five per cent of the patients had reactions of less than 5 mm. of induration, although they had roentgenographic and bacteriologic evidence of tuberculosis. An additional 11 per cent had reactions ranging from 5 to 8 mm., and the remaining 84 per cent had reactions of 9 mm. or more.

Deviations: Deviations from the assigned treatment regimens as shown in table 3 are broadly classified as: tuberculous: either death or critical worsening; intolerance to one or more of the therapeutic agents; and extraneous: those apparently unrelated to the patient's disease or his chemotherapy, such as leaving the hospital, refusing therapy, nontuberculous illness or death, and administrative error. Tuberculous or drug-intolerance deviations will be considered in the analysis of the effectiveness of the regimens.

Of the 1,882 patients entering the trial, 228 (12 per cent) failed to complete the first course of treatment for extraneous reasons. Another 263 (18 per cent) of the 1,492 patients who started the second course were lost similarly. Extraneous deviations are undesirable but, in trials extending over many months, they appear to be unavoidable. Their minimal effect is to reduce the number of patients available for analysis. At worst, they may seriously distort the comparability achieved

TABLE 3

DISPOSITION OF PATIENTS ON EACH REGIMEN DURING 32 WEEKS OF TREATMENT

	Total	Regimen				
		1	2	3	4	5
Total entering trial	1,882	384	368	380	369	381
Deviated from first course	390	79	55	72	80	104
Extraneous*	228	55	29	39	50	55
Tuberculous†	27	5	6	6	7	3
Intolerance	135	19	20	27	23	46
Total entering second course	1,492	305	313	308	289	277
Deviated from second course	331	63	78	76	62	52
Extraneous*	263	59	63	58	47	36
Tuberculous†	8	1	1	3	1	2
Intolerance	60	3	14	15	14	14
Completed assigned therapy	1,161	242	235	232	227	225
Available for evaluation of toxicity during first course‡	1,654	329	339	341	319	326
Available for evaluation of toxicity during second course§	1,229	246	250	250	242	241
Available for therapeutic evaluation‖	1,196	248	242	241	235	230

* Includes these reasons: left hospital, refused therapy, nontuberculous illness or death, and administrative error.

† Includes tuberculous death and critical worsening.

‡ Total entering the trial *less* those deviating from first course for extraneous reasons.

§ Total entering second course *less* those deviating for extraneous reasons.

‖ Total completing assigned therapy *plus* those who deviated for tuberculous reasons.

initially by the chance allocation of therapy. Fortunately, in the present trial, they were similar in frequency and kind on each of the regimens, nearly three-fourths being departures from the hospital, usually against advice.

Results

Toxicity: When a patient developed signs or symptoms of drug intolerance, the suspected drug or drugs were temporarily discontinued. After the symptoms subsided, the patient was usually given a test dose of the suspected drug and, if intolerance was again observed, the drug was permanently discontinued and other suitable chemotherapy substituted. When the intolerance was severe or potentially serious, such as liver damage, the offending agent was not tried again. This analysis has been limited to severe or persistent instances of intolerance for which the drug was permanently stopped or very markedly reduced.

Isoniazid: Only 14 patients (0.9 per cent) of a total of 1,492 were unable to tolerate a sixteen-week course of isoniazid. Twelve patients had allergic reactions manifested by fever and rash, one developed mild peripheral neuritis, and one died of toxic hepatitis that may have been produced by either isoniazid or PAS. Only one instance of drug intolerance, an allergic reaction, was observed among 246 patients while they were receiving a second sixteen-week course of isoniazid.

Para-aminosalicylic acid: Intolerance was severe enough to require a change in treatment in 74 (5.3 per cent) of the 1,401 patients who received a sixteen-week course of PAS. Forty of the 74 patients had allergic reactions to PAS, 13 changed treatment because of severe gastrointestinal symptoms, and 19, although unable to continue the full dose of PAS because of gastrointestinal complaints, did continue with one-half the usual dose.

Two patients developed hepatitis. One, as mentioned above, died of toxic hepatitis while receiving isoniazid—PAS. The other had hepatitis with jaundice; his symptoms subsided when isoniazid—PAS was discontinued and did not recur when isoniazid was given subsequently. It is likely that this was a reaction to PAS although the possibility of infectious hepatitis cannot be excluded.

Para-aminosalicylic acid toxicity did not appear to be related to the age, race, sex, or body weight of the patients. Intolerance tended to occur rather early in treatment. In contrast to the 5 per cent rate during a sixteen-week course, only 2 (0.8 per cent) of the 246 patients continuing on PAS failed to tolerate it during their second sixteen-week course. Both patients had gastrointestinal symptoms.

Streptomycin: When streptomycin was given in a dose of 1 gm. twice a week, intolerance was observed in only 18 (2.2 per cent) of 819 patients. Toxicity occurred five times more frequently among patients receiving 1 gm. of streptomycin daily, 37 (11.3 per cent) of 326 patients. As shown below, there was a twelvefold difference in the incidence of vestibular dysfunction manifested by dizziness and tinnitus and a threefold difference in the frequency of allergic reactions.

Type of Intolerance	One Gram Twice a Week		One Gram Daily	
	Number	Per Cent	Number	Per Cent
Total treated	819	—	326	—
Vestibular dysfunction	4	0.5	19	5.8
Allergic reaction	13	1.6	17	5.2
Other	1	0.1	1	0.3

The number of patients unable to tolerate daily streptomycin was large enough so that the frequency of toxicity could be examined in relation to various characteristics of the patients. Intolerance was related to race and sex as shown in the following text table.

Sex and Race	Total	Vestibular Dysfunction		Allergic Reaction	
		Number	Per Cent	Number	Per Cent
Males					
White	139	6	4.3	7	5.0
Nonwhite	56	1	1.8	1	1.8
Females					
White	78	11	14.1	7	9.0
Nonwhite	53	1	1.9	2	3.8

Both vestibular involvement and allergic reactions were more frequent in women than in men and, for each sex, more frequent in white than in nonwhite patients. Further analysis suggested that patients more than fifty-five years old were at a slightly greater risk of strepto-

mycin toxicity, but no association was found with body weight or stage of disease.

Pyrazinamide: Of a total of 1,236 patients treated with pyrazinamide in combination with streptomycin or isoniazid, 58 patients (4.7 per cent) were unable to tolerate the drug. Forty-eight patients developed hepatitis, which was fatal for 2 patients, a 46-year-old Negro male and a 53-year-old white female. The remaining 10 had some other type of reaction (7 allergic, 2 gastrointestinal, and one aphthous ulcer of the lip).

The risk of hepatitis was not related to body weight or stage of disease. Analysis of sex–race data disclosed an apparent association with sex but not with race, as shown in the text table below:

Sex and Race	Total	Toxic Hepatitis	
		Number	Per Cent
Males			
White	546	25	4.6
Nonwhite	225	13	5.8
Females			
White	278	6	2.2
Nonwhite	187	4	2.1

The sex difference was in part related to age, particularly because of the absence of any cases among women less than thirty-five years of age, as shown below:

Sex and Age	Total	Toxic Hepatitis	
		Number	Per Cent
Males			
Less than 35 years	186	5	2.7
35–54 years	375	16	4.3
55 years or older	209	17	8.1
Females			
Less than 35 years	238	—	—
35–54 years	165	7	4.2
55 years or older	61	3	4.9

The 48 cases of hepatitis have been divided into four categories according to degree of *severity*. While not influenced by age, sex, race, body weight, or stage of disease, the severity of the hepatic episode was directly related to the duration of treatment. Although instances of hepatitis of each degree of severity occurred from the fourth through the sixteenth week of treatment, patients with the most severe hepatitis had, on the average, received pyrazinamide for five weeks longer than those with the mildest episodes, as shown in the table below:

	Severity of Reaction			
	Marked	Moderate	Mild	Very Mild
Number of cases	19	10	11	8
Average weeks of treatment	12.4	10.3	9.5	7.2

Toxicity of combinations: The number of patients unable to tolerate sixteen-week courses of each combination of antimicrobial agents is shown below. Except for isoniazid plus pyrazinamide, the frequency of patients unable to tolerate a combination is somewhat less than the sum of those with toxic reactions to each drug individually, because a few patients were unable to take either drug. Toxicity was least common on isoniazid—PAS and most common on daily streptomycin—pyrazinamide.

Drug Combination	Number of Patients Taking Combination	Unable to Tolerate	
		Number	Per Cent
Isoniazid—PAS			
During first 16 weeks	1,151	67	5.8
During second 16 weeks	246	3	1.2
Isoniazid—pyrazinamide	341	27	7.9
Streptomycin (biweekly)—PAS	250	15	6.0
Streptomycin (biweekly)—pyrazinamide	569	37	6.5
Streptomycin (daily)—pyrazinamide	326	46	14.1

Toxicity of regimens: The rates shown in the text table above for each combination have been used to determine the incidence of toxic reactions which may be expected on each regimen. For this purpose, it is assumed that no deviations for extraneous reasons occur. Isoniazid—PAS for thirty-two weeks had the lowest rate of drug intolerance, 6.9 per cent. On *Regimens 2, 3* and *4*, which all used the same four drugs in the same dosages but in different combinations, the rates

were approximately equal, ranging from 11.9 to 13.4 per cent. However, the higher toxicity rate of daily streptomycin raised the rate of intolerance to 19.1 per cent on regimen 5.

The proportion of patients whose films were interpreted as showing substantial (moderate or marked) improvement was similar for all regimens. *Regimen 5*, daily streptomycin—pyrazinamide switching to isoniazid—PAS had a slight, though not statistically significant, superiority. At the end of thirty-two weeks of treatment the proportion of patients with substantial improvement ranged from 88 per cent on *Regimen 5* to 81 per cent on *Regimen 1*. The differences among regimens were somewhat greater when only marked improvement was considered. *Regimen 5* was most effective, 57 per cent of the patients showing marked improvement at the end of thirty-two weeks, and *Regimen 1* was least effective, 43 per cent showing marked improvement.

Number	Regimen		Per Cent Unable to Tolerate Regimen
	First Course	Second Course	
1	Isoniazid—PAS	Isoniazid—PAS	6.9
2	Isoniazid—PAS	Streptomycin (biweekly)—pyrazinamid	11.9
3	Isoniazid—pyrazinamide	Streptomycin (biweekly)—PAS	13.4
4	Streptomycin (biweekly)—pyrazinamide	Isoniazid—PAS	11.9
5	Streptomycin (daily)—pyrazinamide	Isoniazid—PAS	19.1

Roentgenographic change: The present material on therapeutic effect excludes patients who could not complete their assigned regimens because of drug intolerance as well as patients who deviated from their assigned regimens for extraneous reasons (table 3). The population available for evaluation of roentgenographic change was further reduced to 1,091 by excluding 50 patients with advanced disease but with no bacteriologic evidence of tuberculosis and 55 patients whose initial cultures had organisms resistant to one or more drugs. The procedures for the interpretation of roentgenographic evidence used to obtain an unbiased and objective comparison of the regimens have been described in an earlier paper from the series (4). The results are shown in figure 6.

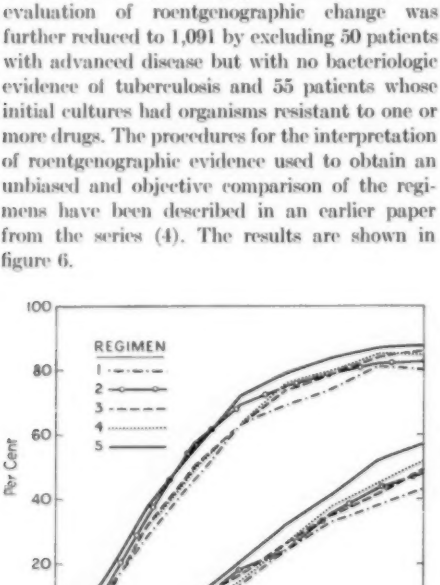

FIG. 6. Percentage of patients showing substantial roentgenographic improvement (upper set of curves) and marked roentgenographic improvement (lower set of curves) on 5 treatment regimens.

Bacteriologic change: While roentgenographic change represents to a large extent the host's response to infection, reversal of infectiousness, as measured by recovery of viable tubercle bacilli in sputum specimens, is chiefly the direct effect of chemotherapy. Thus, change in infectiousness is a most important measure in comparing chemotherapeutic regimens.

The bacteriologic changes among 960 patients with initially positive drug-susceptible cultures are shown in figure 7. On all regimens, reversal of infectiousness continued throughout the first twenty-four weeks with little change thereafter. The curves for *Regimens 1*, *2*, and *3*, all of which used isoniazid for the first sixteen weeks, were very similar throughout treatment except that patients did slightly less well on *Regimen 2*, after biweekly streptomycin—pyrazinamide was substituted for isoniazid—PAS. At the end of thirty-two weeks, 11.6 per cent of the patients on this regimen were still infectious, compared with 8.4 per cent on *Regimen 1* and 8.7 per cent on *Regimen 3*.

Results on the two regimens with an initial course of streptomycin—pyrazinamide were

strikingly different. *Regimen 4*, in which streptomycin was given twice a week, appeared to be nearly as effective as the initial isoniazid regimens for the first twelve weeks of treatment. Because there was practically no further change during the next four weeks, this regimen was decidedly inferior by the sixteenth week, when 42 per cent of the patients were still infectious, twice as many as on the three isoniazid regimens. Substitution of isoniazid—PAS produced a rapid bacteriologic change, and by the twenty-fourth week as few patients remained infectious on this regimen as on *Regimens 1*, *2*, and *3*.

Regimen 5, in which the initial course was daily streptomycin—pyrazinamide, produced more rapid reversal of infectiousness than any other regimen. After twelve weeks of treatment, only 25 per cent of the patients were still excreting tubercle bacilli. Similar to patients on *Regimen 4*, very few additional patients became sputum negative during the next four weeks, and by the sixteenth week this regimen had lost its advantage. With the switch to isoniazid—PAS, reversal of infectiousness again occurred, and less than 5 per cent were still infectious after thirty-two weeks. This regimen had an early advantage and a slight, though not statistically significant, superiority at the end of the treatment period.

Drug susceptibility: All cultures initially positive for tubercle bacilli and, with the exception of those from one hospital, all cultures positive during treatment were sent to the Trudeau Laboratory where they were tested under the direction of Dr. William Steenken for susceptibility to each of the four drugs used in the study. The laboratory workers had no knowledge of the treatment any patient was receiving.

Susceptibility to each drug was tested in the following concentrations:

Drug	Concentration in γ per Ml.
Streptomycin	100, 10
Isoniazid	10, 5, 1.0, 0.2, 0.1
Pyrazinamide	500, 100, 20, 10
PAS	100, 10, 1.0

Growth in each tube was read every week during six weeks of incubation and compared with growth in a control tube. The amount of growth was recorded as follows: actual colony count if less than 50; 1+ = 50 to 200 colonies; 2+ = more than 200 colonies but not uncountable; 3+ = innumerable but not confluent colonies; 4+ = confluent growth over the entire surface.

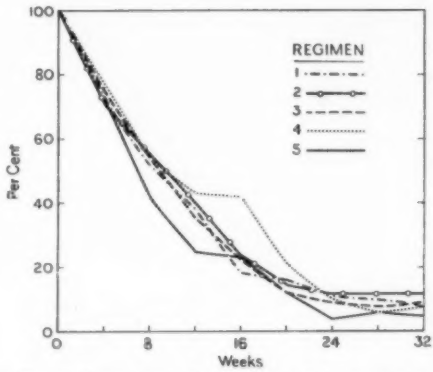

FIG. 7. Percentage of cultures positive for tubercle bacilli among patients treated on 5 regimens.

Growth present after five weeks of incubation has been accepted in the present analysis as representing the degree of resistance to each drug. Growth in the control tube was almost invariably 4+; when it fell below 3+, the test was repeated. The lower limit of significant resistance has been arbitrarily defined as 3+ growth in 10 γ of streptomycin, 1+ growth in 0.2 γ of isoniazid, 1+ growth in 10 γ of PAS, and 1+ growth in 100 γ of pyrazinamide.

Of the 1,882 patients who entered the trial, 1,530 had initially positive cultures tested for susceptibility. As defined above, 3.0 per cent of these cultures were resistant to streptomycin, 1.2 per cent to isoniazid, 0.7 per cent to PAS, and 0.9 per cent to pyrazinamide. The following analysis of drug susceptibility is based on 881 patients who had organisms initially susceptible to all four drugs and who completed thirty-two weeks of assigned treatment.

Pyrazinamide: Bacterial resistance has been the generally accepted explanation for the failure of pyrazinamide as a single drug. *A priori*, the absence of bacteriologic change between the twelfth and sixteenth weeks on both *Regimens 4* and *5* in which streptomycin was combined with pyrazinamide might be attributed to the loss of drug susceptibility to pyrazinamide. The results presented below reflect the inadequacy of this explanation, since many of the cultures were still susceptible to pyrazinamide—streptomycin in the sixteenth week.

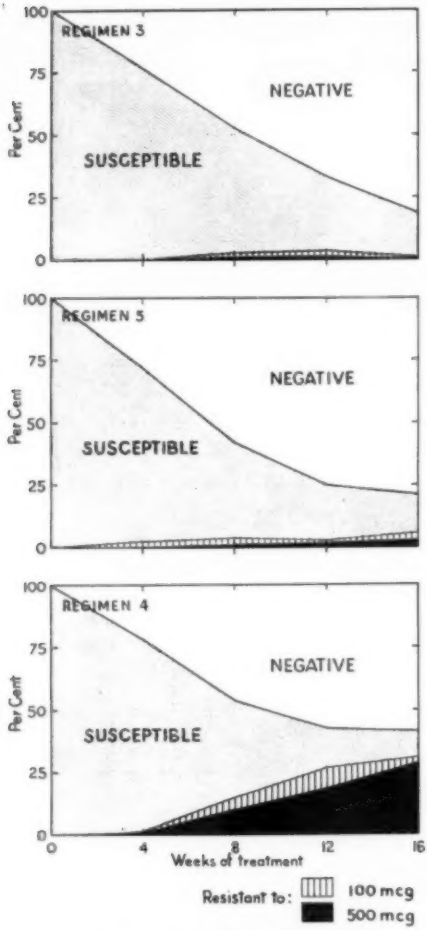

FIG. 8. Results of pyrazinamide-susceptibility tests during sixteen weeks of treatment with pyrazinamide — isoniazid, pyrazinamide — daily streptomycin, and pyrazinamide—bi-weekly streptomycin.

The emergence of resistance to pyrazinamide is shown in figure 8 for the three regimens in which this drug was used in the first sixteen-week course. Resistance to pyrazinamide began to emerge after four weeks of treatment. At the end of sixteen weeks, only 1.4 per cent of the patients on *Regimen 3* and 6.4 per cent on *Regimen 5* had bacilli resistant to at least 100 γ of pyrazinamide. When this drug was combined with biweekly streptomycin, organisms resistant to pyrazinamide appeared in 31.3 per cent of the patients after sixteen weeks.

Streptomycin: Streptomycin resistance is shown in similar charts in figure 9 for the two regimens in which this drug was combined with pyrazinamide during the first sixteen weeks of therapy. Beginning in the fourth week of treatment, the proportion of patients with streptomycin-resistant organisms gradually increased to 10.5 per cent on *Regimen 5* and to 9.6 per cent on *Regimen 4* by the sixteenth week. Resistance to the 100-γ concentration of streptomycin was much more frequent with daily streptomycin than with the biweekly dose.

Isoniazid: Resistance to isoniazid appeared more frequently when the drug was combined with pyrazinamide than when combined with PAS, as shown in figure 10. Resistance to at least 0.2 γ of isoniazid began to appear after four weeks of treatment on both regimens. After sixteen weeks of treatment with isoniazid—PAS, only 2.0 per cent of the patients had bacilli resistant to isoniazid in contrast to 10.8 per cent

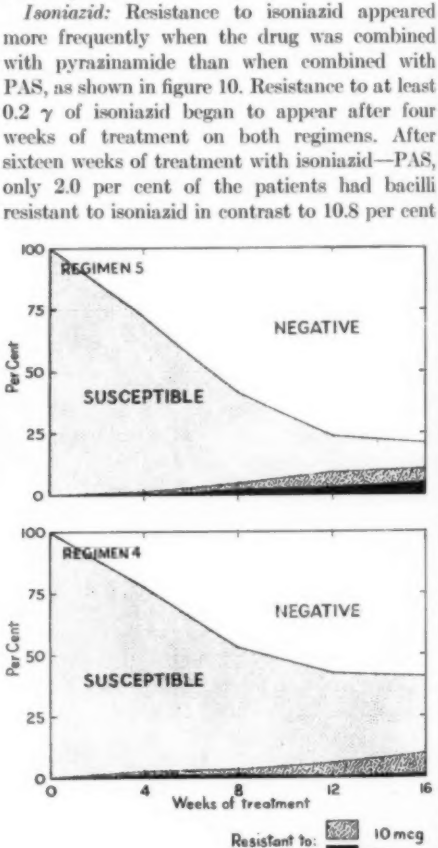

FIG. 9. Results of streptomycin-susceptibility tests during sixteen weeks of treatment with daily streptomycin—pyrazinamide and with biweekly streptomycin—pyrazinamide.

of the patients who received isoniazid—pyrazinamide.

Para-aminosalicylic acid: Bacilli resistant to PAS, except for occasional slight growth in 1.0 γ, did not appear in any patient during the first sixteen weeks of treatment with isoniazid—PAS.

Combined resistance: When treatment was changed after sixteen weeks, reversal of infectiousness occurred as frequently among patients with organisms resistant to drugs given in the first course as among patients with susceptible organisms. At the end of thirty-two weeks of treatment in which four different drugs were given in paired combinations, none had bacilli resistant to all four drugs and very few to three drugs.

Discussion

In a continuing search for the optimal antimicrobial therapy, the United States Public Health Service Tuberculosis Therapy Trials have tested new treatment regimens against the best current treatment. In a trial in 1953, isoniazid—PAS emerged as the most effective regimen and in subsequent trials continued to excel other treatments. As the best regimen then available, it was used as the control regimen in the present trial.

It is apparent from the results presented that *Regimens 2*, *3*, and *4* had no therapeutic advantage over isoniazid—PAS (*Regimen 1*) and produced twice as many toxic reactions. Furthermore, all three regimens had the added disadvantages of an injected drug, streptomycin, and the most expensive and potentially dangerous drug, pyrazinamide. It would seem, therefore, that none of these regimens should be seriously considered for initial treatment although some combinations might have a use in the chemotherapy of treatment failures.

Regimen 5, daily streptomycin—pyrazinamide followed by isoniazid—PAS, has, of course, the same disadvantages. Even more patients were unable to tolerate this regimen, three times as many as on continuous isoniazid—PAS. However, in contrast to the other three regimens using all four drugs, *Regimen 5* was therapeutically superior to isoniazid—PAS in the present trial.

In the text table below, an attempt has been made to compare these two regimens by taking into consideration both drug intolerance and therapeutic effectiveness. It has been assumed

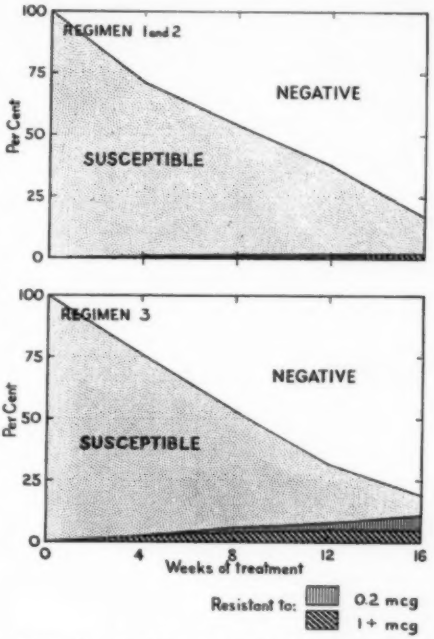

Fig. 10. Results of isoniazid-susceptibility tests during sixteen weeks of treatment with isoniazid—PAS and isoniazid—pyrazinamide.

that 1,000 patients would start treatment on each regimen and that none would deviate for extraneous reasons. The number of patients able to tolerate each thirty-two-week regimen and the number unable to tolerate one or more drugs in the regimen are derived from the percentages in an earlier table on drug intolerance. The number of patients who would still be infectious after thirty-two weeks of treatment among those able to tolerate each regimen corresponds with the information in figure 7. The number of patients who would remain infectious among those with drug intolerance is based on the experience of patients with toxic reactions in the trial. When only one antituberculous drug was available, development of toxicity often meant the end of antimicrobial treatment for a patient. However, with four drugs available, intolerance to one drug or combination of drugs in the present trial resulted only in the substitution of another drug or combination. Thus, as the results indicate, toxicity no longer carries the added penalty of deprivation of effective therapy. As shown in the

final line, among the total number of patients beginning treatment, twice as many would still be infectious after thirty-two weeks on *Regimen 1* as on *Regimen 5*.

	Regimen	
	1	5
Number assumed to start treatment	1,000	1,000
Able to tolerate regimen	931	809
Infectious at 32 weeks	78	40
Unable to tolerate regimen	69	191
Infectious at 32 weeks	20	5
Per cent infectious at 32 weeks	9.8	4.5

This material suggests that daily streptomycin—pyrazinamide followed by isoniazid—PAS is an extremely effective regimen for initial treatment of pulmonary tuberculosis. However, the potentially serious toxicity of pyrazinamide, the higher cost of the drugs, and the inconvenience of daily streptomycin injections produces some hesitation in its indiscriminate use in all previously untreated patients.

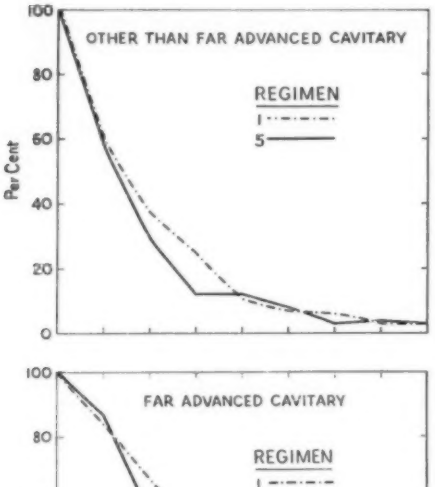

FIG. 11. Percentage of cultures positive for tubercle bacilli during thirty-two weeks of treatment with isoniazid—PAS and with daily streptomycin—pyrazinamide followed by isoniazid—PAS, among patients divided by type of disease on admission.

In this connection, the bacteriologic change on *Regimens 1* and *5* has been examined by type of disease. Bacteriologic change among patients with other than far advanced cavitary disease is shown in the upper chart in figure 11. Reversal of infectiousness occurred very rapidly with little difference between the two regimens, except for a temporary advantage of *Regimen 5* in the early weeks of treatment. For this type of disease, there was no therapeutic superiority of *Regimen 5* to compensate for the increased risk of toxicity.

Bacteriologic change was slower among patients with far advanced cavitary disease, as shown in the lower chart. Infectiousness was reversed more frequently on *Regimen 5* with only 38 per cent of the patients still infectious after twelve weeks of treatment compared with 50 per cent on *Regimen 1*. By sixteen weeks the relative positions of the two regimens were reversed. However, after treatment was changed to isoniazid—PAS, *Regimen 5* regained its advantage. After thirty-two weeks only 6.8 per cent of the patients on this regimen were still infectious compared with 14 per cent on continuous isoniazid—PAS.

It would appear, therefore, that daily streptomycin—pyrazinamide followed by isoniazid—PAS is a promising regimen for patients with far advanced cavitary disease. It is precisely for these patients with a generally less favorable prognosis that the therapeutic benefit would outweigh the risk of drug intolerance.

In the results on bacteriologic change, it was observed that with daily streptomycin—pyrazinamide reversal of infectiousness occurred rapidly during the first twelve weeks of treatment with virtually no further change in the next four weeks (*see* figure 7). Although this hiatus could be only partially explained by the emergence of drug-resistant organisms, continued treatment in the presence of what might be called "clinical resistance" would seem futile. The results that could be expected among patients with far advanced cavitary disease if the second course, isoniazid—PAS, was started

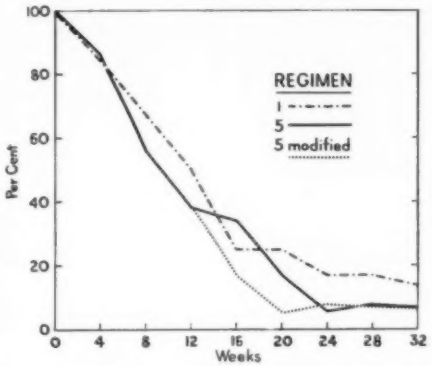

FIG. 12. Observed percentage of cultures positive for tubercle bacilli among patients with far advanced cavitary disease treated with *Regimen 1* (isoniazid—PAS for thirty-two weeks), *Regimen 5* (daily streptomycin—pyrazinamide for sixteen weeks followed by isoniazid—PAS for sixteen weeks), and expected percentage with modified *Regimen 5* (daily streptomycin—pyrazinamide for twelve weeks followed by isoniazid—PAS for twenty weeks).

at the end of twelve weeks are shown in figure 12. The reversal of infectiousness observed for *Regimen 5* in figure 11 after a change of drugs at sixteen weeks would presumably occur four weeks earlier. Thus modified, *Regimen 5* should retain its early advantage throughout thirty-two weeks.

A shortened initial course of daily streptomycin—pyrazinamide could also be expected to decrease drug intolerance. Of the 37 patients in this trial who were unable to tolerate sixteen weeks of daily streptomycin, 6 showed intolerance only after the twelth week. With pyrazinamide, only 4 of the 19 mild hepatic reactions occurred after the twelth week of treatment. On the other hand, 14 of the 29 instances of severe hepatitis were detected between the twelth and sixteenth weeks of treatment, although in at least some patients a disturbance in hepatic function had probably begun earlier. It appears, therefore, that limiting pyrazinamide therapy to twelve weeks would effect some reduction in the number of hepatic episodes and would almost certainly modify the extent of many reactions. Lengthening the second course to twenty weeks should not result in any marked increase in intolerance to isoniazid—PAS.

By limiting initial treatment with the regimen to patients with far advanced cavitary disease and reducing the initial course to twelve weeks, most hospitals would have adequate facilities for at least one weekly test of liver function and careful observation of patients for symptoms of hepatic disturbance. These precautions should reduce the risk of serious toxic reactions and make practicable an extremely effective therapeutic regimen, daily streptomycin—pyrazinamide switching to isoniazid—PAS.

SUMMARY

A United States Public Health Service Tuberculosis Therapy Trial among 1,882 patients in twenty-five hospitals investigated the sequential use of pairs of antituberculous drugs. Various pairings of isoniazid, streptomycin, PAS, and pyrazinamide in two sixteen-week courses were tested against a thirty-two-week course of isoniazid—PAS. Clinical, bacteriologic, and roentgenographic examinations were made at four-week intervals throughout treatment. All cultures of tubercle bacilli obtained were tested in one laboratory for susceptibility to all four drugs.

Three regimens, (*1*) isoniazid—PAS switching to streptomycin twice weekly with pyrazinamide, (*2*) isoniazid—pyrazinamide switching to streptomycin twice weekly with PAS, and (*3*) streptomycin twice weekly with pyrazinamide switching to isoniazid—PAS, showed no therapeutic superiority over a thirty-two-week course of isoniazid—PAS and produced twice as many toxic reactions.

The other regimen tested, daily streptomycin—pyrazinamide switching to isoniazid—PAS, was therapeutically superior to continuous isoniazid—PAS, although three times as many patients were unable to tolerate one or more of the drugs used. For patients with far advanced cavitary disease, this regimen's higher rate of drug intolerance was outweighed by its therapeutic advantage. It would appear that only twelve weeks of daily streptomycin—pyrazinamide followed by twenty weeks of isoniazid—PAS would be less toxic and therapeutically even more effective for these patients.

SUMARIO

Empleo Consecutivo de Combinaciones Apareadas de Isoniacida, Estreptomicina, Acido Para-Aminosalicílico y Piracinamida

En una prueba de terapéutica antituberculosa del Servicio de Sanidad Pública de los Estados Unidos se investigó entre 1,882 enfermos de 25 hospitales el uso consecutivo de pares de drogas

antituberculosas. Se comprobaron combinaciones apareadas de isoniacida, estreptomycina, PAS y piracinamida en dos series de dieciséis semanas contra una serie de treinta y dos semanas de isoniacida—PAS. Se ejecutaron exámenes clínicos, bacteriológicos y radiográficos a plazos de cuatro semanas durante todo el tratamiento. Todos los cultivos positivos fueron estudiados en un laboratorio en cuanto a susceptibilidad a todas las cuatro drogas.

Tres regímenes, (*1*) isoniacida—PAS virando a estreptomicina dos veces semanales con piracinamida, (*2*) isoniacida-piracinamida virando a estreptomicina dos veces semanales con PAS y (*3*) estreptomicina dos veces semanales con piracinamida virando a isoniacida—PAS, no revelaron la menor superioridad terapéutica sobre una serie de treitna y dos semanas de isoniacida—PAS y produjeron el doble de reacciones tóxicas.

El otro régimen ensayado, estreptomicina-piracinamida diariamente virando a isoniacida—PAS, resultó superior terapéuticamente a la isoniacida—PAS continua, aunque tres veces más enfermos se mostraron incapaces de tolerar una o mós de las drogas usadas. Para los sujetos con enfermedad cavitaria muy avanzada, la tasa mós alta de intolerancia medicamentosa del régimen quedó contrapesada por la ventaja terapéutica. Para estos enfermos, parece que doce semanas de estreptomicina-piracinamida diariamente seguidas de veinte semanas de isoniacida—PAS serían menos tóxicas y terapéuticamente hasta más eficaces.

Resume

Emploi consécutif d'associations d'isoniazide, de streptomycine, d'acide para-aminosalicylique et de pyrazinamide

L'"United States Public Health Service" a expérimenté, dans le traitement de 1,882 tuberculeux, répartis dans 25 hôpitaux, l'emploi d'associations de groupes de médicaments antituberculeux. Les différentes combinaisons par pairs d'isoniazide, streptomycine, PAS et pyrazinamide, administrées en deux séries de 16 semaines, ont été comparées à une série de traitement d'isoniazide—PAS, durant 32 semaines. Des examens cliniques, bactériologiques et radiographiques furent effectués à intervalles de quatre semaines pendant tout le traitement. Des tests de sensibilité aux quatre produits furent effectués en laboratoire pour tous les cas à culture positive.

Il a été constaté que trois modes d'administration, (1) isoniazide—PAS, alternés deux fois par semaine avec streptomycine et pyrazinamide; (2) isoniazide-pyrazinamide, alternées deux fois par semaine avec streptomycine—PAS, et (3) streptomycine-pyrazinamide, alternées avec isoniazide—PAS, n'avaient pas produit de meilleurs résultats thérapeutiques et avaient provoqué deux fois plus de réactions toxiques, qu'un traitement de 32 semaines par l'association isoniazide—PAS.

L'autre méthode, streptomycine-pyrazinamide quotidiennes, alternées avec l'isoniazide—PAS, a donné de meilleurs résultats que l'emploi continu d'isoniazide—PAS, bien que trois fois plus de malades n'ont pu tolérer l'un ou plusieurs des produits employés. Chez les malades atteints de cavernes avancées, ce taux élevé d'intolérence aux médicaments était compensé par les résultats thérapeutiques obtenus. Il semble qu'un traitement journalier de 12 semaines par streptomycine-pyrazinamide, suivi d'isoniazide—PAS pendant 20 semaines, soit moins toxique et plus efficace pour les malades.

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VII. COMPARISON OF ISONIAZID ALONE WITH ISONIAZID—PAS IN THE ORIGINAL CHEMOTHERAPY OF NONCAVITARY PULMONARY TUBERCULOSIS[1, 2]

Results from the Veterans Administration-Armed Forces Co-operative Study on the Chemotherapy of Tuberculosis

SAMUEL PHILLIPS

(Received for publication June 1, 1959)

Introduction

The use of isoniazid alone in the treatment of pulmonary tuberculosis has been a controversial subject almost since the drug's introduction in 1952. Following the preliminary investigations (1) which indicated that isoniazid possessed marked therapeutic value in tuberculosis, the Veterans Administration-Armed Forces Co-operative Study on the chemotherapy of tuberculosis instituted a limited "pilot" study in patients with all stages of active tuberculosis, employing isoniazid alone in doses of either 150 mg. or 300 mg. daily in the treatment of randomized cases. After a trial of less than one year the data at the Twelfth Chemotherapy Conference, held in 1953 at Atlanta, Georgia, indicated that isoniazid alone probably was somewhat inferior therapeutically to "combined therapy" in the patients studied under the terms of the protocol (2). What seemed alarming, however, was that more than half the patients whose sputum still remained positive for tubercle bacilli at the end of four months' therapy with isoniazid alone excreted tubercle bacilli that were found to be resistant to isoniazid *in vitro* (3). These findings persuaded the majority of the members of the Twelfth Conference to recommend that the further study of isoniazid alone be abandoned.

One year later, at the Thirteenth Veterans Administration-Armed Forces Conference, a series of patients treated with isoniazid alone in a study conducted by the U. S. Public Health Service (*4a*), was presented by Eckley (*4b*), as a part of a strictly objective roentgenographic investigation of various chemotherapeutic regimens which had been administered to randomized patients for forty weeks. He pointed out that the significant roentgenographic improvement which was obtained with isoniazid alone was approximately equal to that which was obtained with various multiple-drug regimens in patients with noncavitary pulmonary tuberculosis, and probably also in patients showing only single cavities. In addition, studies (5, 6) by other investigators indicated that good therapeutic results could be obtained with isoniazid alone in patients with previously untreated pulmonary tuberculosis. The Cornell group of investigators (5) was sufficiently impressed with their results to question the need for the prevailing practice of insisting upon multiple-drug therapy irrespective of the circumstances.

At the Fourteenth Conference in 1955, Tucker (7) reviewed the Veterans Administration-Armed Forces data on isoniazid alone. With the more complete information then available, a detailed examination of the various factors involved led him to conclude that the minimal and the noncavitary cases in the initial pilot studies had responded probably equally as well to isoniazid alone as to streptomycin—PAS. This was in contrast to the patients with the more advanced, cavitary disease. He expressed the opinion that: "We are much closer to the point where we may by suitable analysis of our data begin to pin-point the chemotherapy of tuberculosis according to the type of disease a patient has."

At the end of the Fourteenth Conference, a random-selection type of study was organized to compare the use of isoniazid alone with isoniazid-PAS in the original treatment of minimal and noncavitary moderately advanced cases of pulmonary tuberculosis. Then, as now, the use of isoniazid alone or isoniazid-PAS represented the only two regimens of major antituberculous drugs that could be given by mouth without undue risk of potentially serious drug toxicity. It is of considerable interest, therefore, to note that the present study, organized in 1955, represented the first attempt to make a concurrent comparison of these

[1] From the Pulmonary Disease Service, Veterans Administration Medical Teaching Group Hospital (Kennedy), Memphis, Tennessee.

[2] Presented in greater part at the Eighteenth Conference on the Chemotherapy of Tuberculosis of the Veterans Administration-Armed Forces, in February, 1959, St. Louis, Missouri.

TABLE 1
COMPARISON OF ISONIAZID ALONE WITH ISONIAZID—PAS
Total Cases Observed

Regimen	0–4 Months	5–8 Months
Isoniazid	134	94
Isoniazid—PAS	148	95
Total	282	189

two regimens by any of the groups in the United States or abroad engaged in large-scale "controlled" tuberculosis chemotherapy evaluations.

As the study was organized, the dose of isoniazid was to be 100 mg. and that of PAS, 4 gm.; both to be given three times daily. The accumulation of cases was very slow, however, and not until the Seventeenth Conference, in 1958, were there sufficient data for even preliminary analysis (8). An examination of the 151 cases studied up to that time indicated that the 65 patients who had been treated with isoniazid alone had apparently done about as well as the 86 who had received isoniazid—PAS. No significant difference could be discovered on the basis of either roentgenographic improvement, reversal of infectiousness, or achievement of "target point." The fear of drug-resistant organisms was not substantiated by the data available.

At the Eighteenth Conference, held February, 1959 at St. Louis, Missouri, the cases of 282 patients were available for analysis, 189 of whom had received treatment for five to eight months (table 1). All cases were randomized. This was accomplished through a sealed "envelope system" in 67 per cent of those on isoniazid and in 59 per cent of those on isoniazid—PAS; in the remainder, the randomization was accomplished through the last digit of the patient's hospital number. The diagnosis in all cases was confirmed by demonstration of the presence of tubercle bacilli in the sputum.

OBSERVATIONS

Background data: A review of the patients on the two regimens showed remarkable similarities (table 2). The proportion of minimal and moderately advanced cases, the incidence of progressive disease, and the occurrence of bilateral lesions were almost identical in the two groups. Other background factors such as race, sex, and prior thoracic surgery were also very similar. However, the isoniazid—PAS group contained more young and less elderly patients (table 3)—a probable slight advantage for the multiple-drug regimen.

There were no deaths during the eight months of this study. Resectional surgery was performed during the zero- to four-month period on 3 patients who were receiving isoniazid alone and on 2 who were receiving isoniazid—PAS. During the five- to eight-month period, 3 patients on isoniazid alone, and 6 on isoniazid—PAS underwent pulmonary resection. Two other patients on isoniazid alone were offered surgery but after the addition of other drugs. Thus, surgical measures to supplement chemotherapy were considered necessary in 7 per cent of the patients treated with isoniazid alone and in 8 per cent of those who received isoniazid—PAS.

Roentgenographic Improvement

Improvement to a marked or moderate degree following treatment was seen on the chest roentgenograms with about equal frequency in both groups of patients (figure 1). It was almost identical in the patients with minimal disease at zero to four months (about 30 per cent) and also at five to eight months (about 40 per cent). In the patients with moderately advanced disease, roentgenographic improvement occurred slightly more frequently in the patients who received isoniazid—PAS in the period between zero and four months (40 as against 31 per cent), but this trend was reversed between five and eight months in favor of the group receiving isoniazid alone (66 as against 55 per cent). Because the patients on the two regimens were so similar in composition and because the number in each category was comparatively small, all of the patients on isoniazid

TABLE 2
COMPARISON OF ISONIAZID ALONE WITH ISONIAZID-PAS
Extent and Trend of Disease, Per Cent

Type	Isoniazid	Isoniazid—PAS
Minimal	46	44
Moderately advanced	54	56
Progressive	54	58
Stationary	39	37
Regressive	7	5
Unilateral	62	60
Bilateral	38	40

TABLE 3
COMPARISON OF ISONIAZID ALONE WITH ISONIAZID-PAS
Age of Patients

Years	Isoniazid	Isoniazid—PAS
Less than 40	35%	46%
40 to 59	33%	31%
60 and older	32%	23%
Unknown	1 patient	1 patient
Total	100%	100%
Total number of patients	134	148

alone were grouped together, and their degree of roentgenographic improvement was compared with that obtained by the group of patients on isoniazid—PAS. It was found that the marked and moderate roentgenographic improvement achieved on each regimen was practically identical (figure 2). Very few patients in this study showed unfavorable roentgenographic changes; three such episodes occurred in patients who were receiving isoniazid alone and one on isoniazid—PAS during the zero- to four-month observation period; only one patient, on isoniazid alone, showed such a change between five and eight months.

Reversal of Infectiousness

Reversal of infectiousness or "sputum conversion" was considered to have occurred only when sputum cultures for tubercle bacilli were negative for at least three consecutive months. In figure 3 it may be seen that about 65 per cent of patients with minimal disease on either treatment lost their infectiousness between zero and four months. Between five and eight months, however, there was a somewhat higher conversion rate on isoniazid—PAS (95 as against 77 per cent). In the patients with moderately advanced disease, the "conversion" rates between zero and four months were again similar (about 65 per cent); but they were higher in the isoniazid alone group between five and eight months, 91 as against 81 per cent for those treated with isoniazid—PAS. The "conversion" results of the entire group of patients on isoniazid alone were compared with those on isoniazid—PAS. The findings show that the incidence of "sputum conversion" with the two regimens was almost identical (figure 4). Only one patient, on isoniazid—PAS, suffered a bacteriologic relapse. His sputum became positive for tubercle bacilli again during the five- to eight-month period.

In an attempt to test further for possible differences in the therapeutic efficacy of each regimen, the data on all of the patients were examined as to the achievement of "target point." This can be defined briefly as that point in treatment at which the sputum becomes free of tubercle bacilli, the roentgenographic abnormalities have become stable, and all tuberculous cavities have closed. Only the first two criteria apply in this

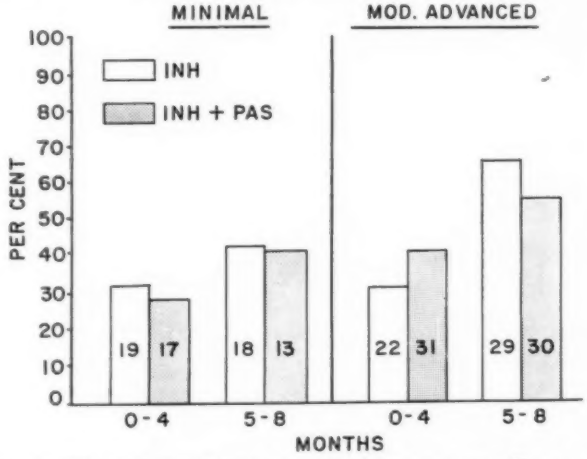

FIG. 1. Roentgenographic change: moderate or marked improvement. Figures on bars represent actual number of patients. INH—isoniazid.

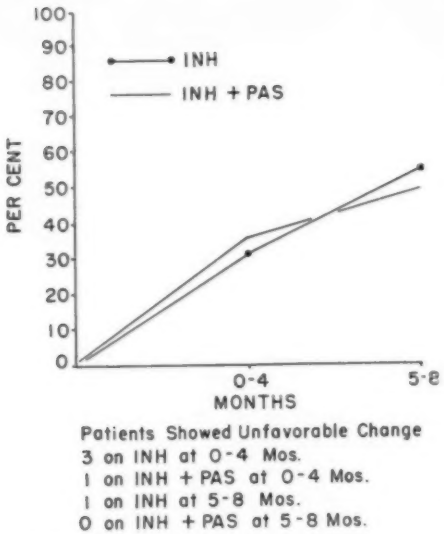

FIG. 2. Roentgenographic change: moderate or marked improvement, all cases. INH—isoniazid.

study, as all of the cases were presumably noncavitary at the start of treatment. Of the patients treated for four months or less, 20 per cent on each regimen reached "target point." For those who had been treated for the period between five and eight months, the time required for achievement of "target point" was likewise determined. It was found (figure 5) that approximately 60 per cent of the patients on each regimen had reached "target point" by the eighth month, and at rates which were practically identical. One patient receiving isoniazid alone, however, suffered a bacteriologic relapse following the achievement of "target point."

Isoniazid Resistance on the Two Regimens

The emergence of strains of tubercle bacilli resistant to isoniazid *in vitro* had been a chief reason for the earlier reluctance to investigate the therapeutic value of isoniazid alone, and therefore the present data are of particular interest. Ninety-four of the 282 patients studied still had tubercle bacilli in the sputum at the zero- to four-month observation point. Only 3 of these 94 patients were discharging organisms that were resistant to isoniazid. Two of these 3 patients were from the group that received isoniazid—PAS. Resistance was defined as bacillary growth equal to the control in a medium containing 1 γ of isoniazid per ml. or the presence of any growth in a medium containing 5 γ of isoniazid per ml. These 3 patients are described in more detail.

Isoniazid alone: N. H. F. had moderately advanced stationary tuberculosis and was treated with isoniazid alone. Sputum obtained after three months of therapy showed confluent growth of tubercle bacilli in an isoniazid concentration of 5 γ per ml. When an exploratory thoracotomy for a suspected pulmonary neoplasm was suggested, the patient left the hospital after only four months of treatment. A roentgenogram at this time showed no change in the tuberculous infiltration.

Isoniazid—PAS: F. F. P. had progressive moderately advanced pulmonary tuberculosis and

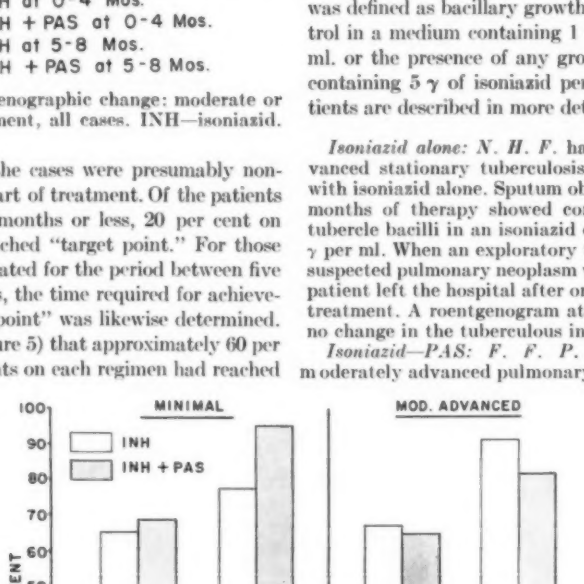

FIG. 3. Reversal of infectiousness (sputum conversion). INH—isoniazid.

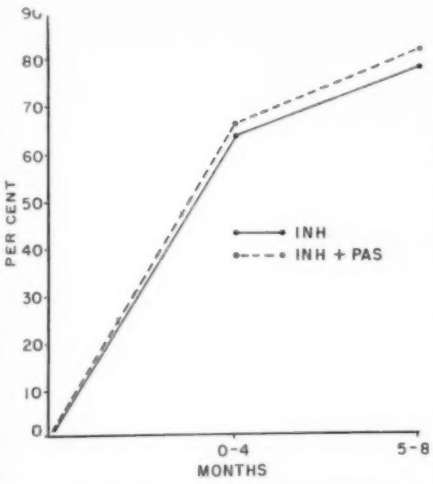

FIG. 4. Reversal of infectiousness—all cases. On isoniazid—PAS, one patient relapsed bacteriologically at five to eight months. INH—isoniazid.

was treated with isoniazid—PAS. After two months of therapy, a sputum specimen revealed tubercle bacilli which showed some ability to grow on medium containing 1 γ of isoniazid per ml. and also in isoniazid concentrations of 5 γ per ml. Although the chest roentgenogram showed moderate improvement after four months of treatment, therapy was changed because of the bacteriologic report. The patient left the hospital through self-discharge soon after, with his sputum still positive for tubercle bacilli.

Isoniazid—PAS: R. C. had moderately advanced stationary pulmonary tuberculosis, and was treated with isoniazid—PAS. During the fourth month of treatment his sputum was found to contain tubercle bacilli which showed some growth in isoniazid concentrations of 1 γ per ml. and 5 γ per ml. Therapy was continued and the sputum became consistently negative for tubercle bacilli by the fifth month. After eight months of therapy, the roentgenogram was considered to have shown moderate improvement. Resective surgery was performed during the ninth month of therapy. Apparently no organisms were isolated from the resected specimen, and the patient was discharged six months later.

Only 28 of the 189 patients treated for five to eight months had not achieved reversal of infectiousness, and in only 3 of the 28 patients were isoniazid-resistant organisms found. Two of the 3 patients were from the group that received isoniazid alone.

Isoniazid alone: K. D. T. had moderately advanced progressive tuberculosis for which he received isoniazid alone. After five months of treatment, a sputum culture showed one isolated colony of *M. tuberculosis* which proved to be resistant to 5 γ of isoniazid per ml. The patient's sputum became negative thereafter and remained negative for tubercle bacilli through to the end of the eight months of treatment. The roentgenogram showed moderate improvement at the end of the study. The above culture was the only culture of tubercle bacilli obtained from this patient from the time treatment was begun.

Isoniazid alone: A. M. T., with minimal progressive pulmonary tuberculosis, received isoniazid alone. His roentgenogram showed worsening after four months of treatment in that the original nodule under treatment, which had been 1 cm. in diameter at the start, had increased to 2 cm. Sputum obtained during the fifth month showed tubercle bacilli which grew on culture medium containing 5 γ of isoniazid per ml. The patient was withdrawn from the study and the therapy was changed to another regimen.

Isoniazid—PAS: G. A. S. had moderately advanced disease, the trend of which was not known, and he was treated with isoniazid—PAS. After seven months of therapy, a sputum specimen revealed tubercle bacilli resistant to 5 γ of isoniazid per ml. The organisms were still susceptible to PAS. After the conclusion of the eight-month study period, streptomycin was added to the regimen, and the lesion at the right apex was resected. The resected specimen showed a small cavity, not previously identified even on pla-

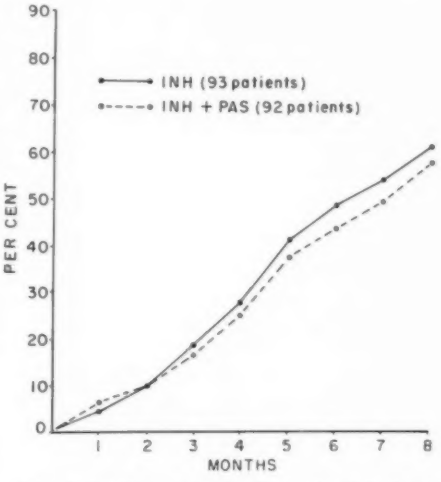

FIG. 5. Achievement of "target point." Patients observed five to eight months. On isoniazid alone, one patient relapsed after achieving "target point." INH—isoniazid.

TABLE 4

COMPARISON OF ISONIAZID ALONE WITH ISONIAZID-PAS

Incidence of Toxicity

Time	0–4 Months		5–8 Months	
Therapy	Isoniazid	Isoniazid—PAS*	Isoniazid	Isoniazid—PAS
Total number of cases	134	148	94	95
Isoniazid	0	1%	0	1%
to PAS	0	11%	0	3%
Both	0	1%	0	0
Total incidence	0	14%	0	4%

* The discrepancy in addition in the column Isoniazid-PAS at 0–4 months is due to the presence of minor fractions.

nigraphy, from which it was possible to culture tubercle bacilli. These were found to be completely susceptible to isoniazid.

From a review of the above data, it would seem that the occurrence of isoniazid resistance was not common in the group of patients studied; it appeared in only 6 patients. It is of particular interest that the emergence of isoniazid-resistant strains occurred equally in the group that received isoniazid—PAS and in the group that received isoniazid alone, and occurred equally on both regimens. Five of the 6 patients with drug-resistant strains had moderately advanced disease, and in the last case described the cavitary lesion may have been present but unidentified at the beginning of the treatment.

It should be reported that, in all of the above patients in whom drug-resistant organisms appeared, drug-susceptibility studies of pretreatment sputum had shown tubercle bacilli to be completely susceptible to the drugs employed. At the same time, it is of interest to note that 2 other patients treated with isoniazid alone and 3 treated with isoniazid—PAS had tubercle bacilli prior to treatment which were able to grow on medium containing 1 γ of isoniazid per ml. although to a lesser extent than on the control. All 5 of these patients responded satisfactorily to their treatment and none developed organisms which became isoniazid resistant by the definition of resistance used in the study.

Drug Toxicity

The incidence of drug toxicity was greater with isoniazid—PAS than it was with isoniazid alone, as would be expected. At zero to four months (table 4) 17 patients (11 per cent) of those on the multiple-drug therapy developed PAS toxicity, consisting mainly of symptoms of gastrointestinal intolerance. Five of the patients developed hypersensitivity reactions, including one patient in whom hepatitis was believed to be a complication of the treatment. None of the patients on isoniazid alone, but 3 of those on isoniazid—PAS therapy developed toxic symptoms, presumably due to isoniazid. There was a febrile reaction in one of these patients, dryness of mouth and dizziness in another, and the development of hepatosplenomegaly in a third. In 10 per cent of the patients on isoniazid—PAS, the toxicity was sufficiently severe to force a change in therapy within the first four months of treatment.

Four patients on multiple therapy experienced symptoms of toxicity during the five- to eight-month period. One developed peripheral neuritis; the other 3 complained of gastrointestinal disturbances. All 4 required changes in therapy. No patient on isoniazid alone experienced toxic symptoms. Although drug toxicity caused losses from the study, the greatest losses occurred through self-discharge of patients from hospitals. At zero to four months, the irregular discharge rate was identical on both regimens, 11 per cent. At five to eight months, it was lower on isoniazid alone (5 per cent) than it was on isoniazid—PAS (11 per cent).

SUMMARY

The data accumulated thus far by means of the Veterans Administration-Armed Forces Cooperative Study appears to indicate that, in patients with minimal or noncavitary moderately advanced disease, the therapeutic results obtained with isoniazid alone are probably equally as good as those obtained with isoniazid—PAS. In the 282 patients' studies for periods up to eight months, the reversal of infectiousness, roentgenographic improvement, achievement of "target point," and the incidence of isoniazid resistance were practically identical in the group treated with isoniazid alone and in the patients who also received PAS. In addition, with isoniazid alone there was an obvious saving in cost and less drug

toxicity than when the isoniazid was accompanied by PAS.

Sumario

Comparación entre la Isoniacida Sola y la Isoniacida—PAS en la Quimioterapia Primitiva de la Tuberculosis Pulmonar No Cavitaria: Resultados del Estudio Cooperativo de la Administración de Veteranos-Fuerzas Armadas sobre la Quimioterapia de la Tuberculosis

Los datos acumulados hasta ahora por medio del Estudio Cooperativo de la Administración de Veteranos y las Fuerzas Armadas parecen indicar que, en los sujetos con enfermedad mínima o moderadamente avanzada no cavitaria, los resultados terapéuticos obtenidos con la isoniacida sola son probablemente tan buenos como los obtenidos con la isoniacida—PAS. En los 282 enfermos estudiados por períodos hasta de ocho meses, el viraje de la infecciosidad, la mejoría radiográfica, el alcance del "blanco" enfocado y la incidencia de resistencia a la isoniacida fueron prácticamente idénticos en el grupo tratado con isoniacida sola y en los enfermos que recibieron además PAS. Agréguese que, con la isoniacida sola, hubo un ahorro manifiesto en el costo y menos intoxicación medicamentosa que cuando la isoniacida iba acompañada del PAS.

Resume

Comparaison entre l'isoniazide et l'isoniazide—PAS dans la chimiothérapie de la tuberculose pulmonaire non-cavitaire: Résultats d'une étude sur la chimiothérapie de la tuberculose, faite conjointement par la "Veterans Administration" et les "Armed Forces"

Les renseignements obtenus jusqu'ici grâce à l'étude faite en collaboration par la "Veterans Administration" et les "Armed Forces," semblent indiquer que chez les malades atteints de lésions minimes ou d'une lésion non-cavitaire modérément active, les résultats thérapeutiques obtenus par l'isoniazide sont probablement similaires à ceux obtenus par l'association isoniazide—PAS. Chez les 282 malades examinés pendant des périodes allant jusqu'à 8 mois, la négativation de l'infection, l'amélioration radiologique, l'obtention du "target point" et la fréquence de l'isoniazido-résistance, étaient pratiquement identiques dans le groupe traité par l'isoniazide seule et dans celui ayant reçu aussi du PAS. De plus, le traitement par l'isoniazide seule était moins coûteux et moins toxique que le traitement combiné isoniazide—PAS.

Acknowledgment

Deep appreciation is expressed to Mrs. Dorothy G. Livings, Statistician, Research Statistics Division, Reports and Statistics Service of the DM&S, Central Office of the Veterans Administration, whose accumulation and tabulation of the data and whose wholehearted cooperation lightened immeasurably the task of the writer.

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THE CHEMOPROPHYLAXIS OF TUBERCULOSIS[1]

A Review

H. P. LAMBERT[2, 3]

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Introduction

Prophylaxis is defined as the prevention of disease. The word may therefore be used to refer to any method of preventing tuberculous disease, whether or not the people for whom the method is used are already infected with tubercle bacilli. The prevention of infection is often known as *primary prophylaxis*, while the prevention of disease in previously infected subjects is known as *secondary prophylaxis*. The possibility of preventing tuberculosis by the use of a chemical agent, as opposed to a vaccination procedure, was raised by the introduction of isoniazid since, with its low toxicity, small cost, ease of administration, and efficacy as an antituberculous agent, this drug fulfills most of the ancillary criteria of an ideal prophylactic. Chemoprophylaxis has been successfully achieved in a number of other infections, such as those caused by malarial parasites and by hemolytic streptococci.

The use of isoniazid to prevent tuberculous disease has been recommended in a number of clinical situations. To decide on the merits of its use, it is necessary to know both the risks of tuberculosis and the effects of the drug in the particular circumstances. It would be especially valuable to know the answers to the following questions:

> How effective is isoniazid in preventing tuberculous disease, and what dose is necessary to do so? In which of the groups at risk is the drug likely to be most effective? What is the effect of its administration on the subject's immunity against tuberculosis? What are the risks and effects of the development of drug resistance? What are the risks of drug toxicity in large populations treated with isoniazid?

A considerable amount of experimental work has now been done in an attempt to answer some of these questions, and several trials of its use in human populations are now under way. It is intended to review this work and to conclude with a tentative outline of the present indications for, and expectations of, isoniazid prophylaxis.

Experimental Work

Perhaps the first relevant experimental work was that of Dubos, Pierce, and Shaefer, published in 1953, soon after the general introduction of isoniazid (1). They were studying antituberculous immunity produced in mice by different types of vaccine and, in one of their experiments, administered isoniazid for periods varying from seven to eighteen days to groups of mice which had been vaccinated with an attenuated strain of *Mycobacterium tuberculosis* R1Rv. The mice were challenged at various times after the end of their isoniazid treatment with a large dose of virulent bacilli, and two weeks after challenge the number of tubercle bacilli in their lungs and spleens was determined. It was shown that the development of immunity as a result of vaccination with the attenuated culture was retarded or prevented by the use of isoniazid. These findings were opposed by those of Bloch and Segal (2), who, in experiments of similar general design, found that the degree of immunity induced by a single intraperitoneal injection of BCG was uninfluenced by giving isoniazid daily for fourteen days, starting on the day of vaccination.

The experimental work on chemoprophylaxis was extended to another species when the first report of the U. S. Public Health Service experiments was published by Ferebee and Palmer (3). Isoniazid was given to guinea pigs in the drinking water in various doses from a month before to ten weeks after challenge with a large dose of virulent tubercle bacilli given by intraperitoneal injection. These experiments showed that a dose of 5 mg. per kg. of body weight per

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day prevented any mortality for at least twenty-six weeks after challenge and that these animals grew at the same rate as the controls. The other main finding was that, after isoniazid-treated tuberculous infection, guinea pigs responded to a later untreated infection very much as they did after vaccination with BCG, and had thus developed some resistance despite the isoniazid treatment.

The conclusions drawn by Bloch and Segal and by Ferebee and Palmer were criticized by Dubos (4) in an editorial in which he pointed out that the infecting doses used were so large as to be capable of inducing some immunity even if multiplication of the organisms in the vaccine had been prevented by isoniazid. When the infecting dose is small—and it nearly always is small in human infections—the administration of isoniazid during the period of infection might well interfere with the development of immunity. This point was tested experimentally by Dubos and Schaefer (5), who produced antituberculous immunity in mice by vaccinating them with a small number, as few as 20 viable units, of virulent bovine tubercle bacilli. Treatment with isoniazid at the time of infection completely prevented the development of immunity induced in this way; but immunity did develop, despite isoniazid treatment, if the primary infecting inoculum was a very large one. The same point was made by Dubos and Pierce (6) in their work on BCG strains when they showed that isoniazid interfered with the development of immunity in mice produced by BCG if the dose of the vaccine was a small one, comparable with that used in human populations.

A number of additional facts about isoniazid prophylaxis in guinea pigs and mice were established in 1956 and 1957. The second report of the U. S. Public Health Service experiments on guinea pigs (7) gave further details of the doses of isoniazid necessary to prevent disease in these animals. Doses of 3 mg. per kg. daily, 5 mg. per kg. three times a week, 10 mg. per kg. twice a week, or 20 mg. per kg. once a week were about equally effective in preventing tuberculosis; but smaller doses than these merely slowed the progress of the disease. Large, less frequent doses were more effective than the same total weekly amount given in small frequent doses. A period of six weeks of isoniazid treatment completely prevented disease, as judged by the death rate and gross pathology at autopsy, during an eight-month observation period after the drug was discontinued. As shown in their previous work, animals reinfected after a period of isoniazid treatment showed considerable immunity, but again, as before, the infecting dose used in both primary and secondary inoculations was a very large one, 5×10^5 organisms. The continued freedom of these animals from tuberculous disease many months after the end of their course of treatment was an especially interesting finding.

Sher and associates (8), who continued similar experiments for a longer period, had less favorable results. They treated guinea pigs with isoniazid for sixty days beginning seven days after infection, and confirmed that the animals remained well for eight months after infection. At this stage, however, the animals began to die at a more rapid rate than the controls and showed morphologic evidence of tuberculosis in their organs. Some of the isoniazid-treated group, however, were killed sixty-seven weeks after infection and showed no evidence of tuberculosis.

A number of interesting facts about chemoprophylaxis in guinea pigs were established by Peizer, Chaves, and Widelock (9) since these workers, as well as studying survival times, also did tuberculin tests, organ cultures, and catalase and isoniazid susceptibility tests. They used two infecting doses, one small and one large, and also varied both the time of starting the isoniazid course and its duration. They showed that isoniazid was highly effective in controlling infection and that this effect was less marked in animals receiving the larger inoculum. Daily isoniazid given for five days before challenge (they administered the drug by intraperitoneal injection) had no advantage over treatment started on the day of infection; but a delay of fourteen days after infection lessened the efficacy of isoniazid. Treatment for fifty weeks gave results no better than treatment for twelve weeks; but a six-week course was less effective. Especially interesting were the results of organ cultures in relation to the tuberculin tests: of 246 animals treated with isoniazid and alive thirty-six weeks after infection, 14 per cent were tuberculin negative, and the organs of autopsied animals in this group were negative on culture. Only 14 per cent of the 211 survivors with persistently positive tuberculin reactions had culturable tubercle bacilli in their organs. Thus,

although the possibility of a very late reactivation could not be entirely excluded, the disease had certainly been very effectively suppressed, especially as a negative tuberculin reaction was maintained for at least twenty-four weeks after the end of treatment in 26 animals. Less reassuring was the fact that, of 126 treated animals from which tubercle bacilli were isolated, 44 per cent harbored organisms with some degree of isoniazid resistance. The effect of chemoprophylaxis in guinea pig tuberculosis has also been studied by Bartmann and his colleagues who, in a series of papers (10), have shown the efficacy of intermittent isoniazid dosage in preventing gross disease and have confirmed the inverse relationship of its effectiveness to the size of the infecting dose.

The action of isoniazid in preventing gross disease had been demonstrated in a different system by Robson and Didcock (11), who showed that the lesion of experimental corneal tuberculosis in the mouse could be prevented by isoniazid and that immunity in the other (uninfected) eye, normally well established at thirty days, failed to develop if the lesion in the infected eye was suppressed by isoniazid. They showed that living tubercle bacilli persisted in the cornea throughout the period of treatment and that the bacilli remained susceptible to isoniazid. The fate of the cornea after isoniazid was discontinued depended on the dose of the drug, and the maximal period of treatment in these experiments was only fifty days.

In summarizing this work on chemoprophylaxis in smaller laboratory animals, it is clear that gross and microscopic tuberculosis can be effectively suppressed during a period of isoniazid administration. This is true not only of experiments in which very large doses of the drug have been used, but also of those in which doses comparable with those used in man have been administered. The fate of the animals after isoniazid treatment has been stopped is much more difficult to analyze and depends on the period of treatment, the timing of isoniazid in relation to the infection, the animal species, and other factors. It is noteworthy, however, that even an animal as susceptible as the guinea pig has often remained free from evidence of tuberculosis for very many months after the end of a period of isoniazid treatment. The animal's state of immunity to a subsequent second challenge clearly depends on the size of initial bacillary inoculum and, if this is small, isoniazid, when given at the time of infection, does interfere with subsequent immunity to a second challenge.

For a number of reasons, however, these animal experiments have a limited relevance to the problems of isoniazid prophylaxis in man. First, the usual difficulties of interpretation deriving from the great species variations in resistance to tuberculosis have to be reckoned with. Second, the dose of isoniazid in these experiments has often been very large; in particular, in those experiments showing the inhibition of antituberculous immunity by isoniazid, animals have been given the drug in doses of 50 to 250 mg. per kg. This inhibition of immunity, as well as the suppression of the progress of the initial infection, might thus have been more marked than if a smaller dose had been used. Third and most important, the time intervals employed in the experiments on mice and guinea pigs are largely irrelevant to the problems of human tuberculosis. Isoniazid has been administered before, at the same time as, or very shortly after the inception of the tuberculous infection, but it is only rarely that the drug is given to man in this time relationship to his infection. In most circumstances in which isoniazid prophylaxis is recommended or considered in man, the subject has already been infected for a long enough period to have a positive tuberculin skin test. The question here is whether an animal or person with a positive tuberculin reaction has already developed to the fullest extent his resistance to tuberculosis or whether resistance continues to increase after the development of skin allergy.

If the development of resistance is incremental over a long period, then isoniazid treatment, while preventing disease at the time it was given, might leave the animal in a less favorable position with regard to its subsequent immunity either to its own remaining tubercle bacilli or to exogenous reinfection. Even if the animal's resistance shows no further increase after the appearance of skin allergy, it must be asked whether the maintenance of whatever resistance has been developed depends on the continued existence of bacilli in the tissues. If these are reduced in number by isoniazid, will the animal's subsequent immunity be less effective than that which accompanies the natural process of healing in a primary complex? Tubercle bacilli in a quiescent state will not, however, necessarily be reduced in number by isoniazid since, like other antimi-

crobial agents, this drug is relatively ineffective against bacteria which are not in a state of active multiplication (12, 13).

This fact emphasizes the fourth way in which the animal experiments do not accurately reproduce the conditions of human infection. In animal experiments the scales may be loaded in favor of isoniazid even when the dose of tubercle bacilli is large, because the drug is set to act, in effect, on a young and therefore susceptible culture introduced into the animal rather than on an old and quiescent population long established there. It must be admitted that for these reasons the work on chemoprophylaxis in smaller animals, while providing a good deal of interesting information, has been limited in its applicability to man. In particular, the question of the development of an animal's immunity after the establishment of infection and the effects of isoniazid on this development have not yet been settled. Experimental work now in progress is aimed at answering these particular questions (14).

Before passing on to the trials of chemoprophylaxis in man, it is necessary to describe Schmidt's important work on monkeys (15*a*) since this avoids many of the disadvantages of the experiments on smaller animals and does bear directly on one possible role of isoniazid prophylaxis in man. Schmidt started with a group of rhesus monkeys, some of which were tuberculin positive (having become infected "naturally") and had roentgenographic evidence of pulmonary tuberculosis, while others were tuberculin negative. Similar numbers of each group of animals were divided among a number of cages, and all of the animals were given isoniazid in their diet for a year. After this period the infected and noninfected monkeys were separated into different areas so that the possibility of subsequent cross-infection was avoided.

The initially uninfected monkeys remained tuberculin negative during their year on isoniazid; 13 of 27 in this group developed tuberculin sensitivity four to eight months after withdrawal of isoniazid, and these animals developed active tuberculosis within two months of this time. The organisms were acquired during the period of isoniazid administration, but in its presence were unable to multiply sufficiently to produce tuberculin sensitivity or disease. After the isoniazid was stopped, the organisms began to multiply in the same manner as after a recent infection, and produced fulminating disease. In sharp contrast was the fate of the 53 animals, originally tuberculin positive and with roentgenographically demonstrable disease, after their year of isoniazid treatment. Five animals had recurrences of their old infections, but the remaining animals remained well for periods of nine to eighteen months and showed no evidence of active tuberculosis when killed. Paradoxically, the severe tuberculosis which would have killed the animals in the absence of chemotherapy had nevertheless evoked immune reactions which, after the monkey's life had been saved by isoniazid, allowed it to remain in good health. The monkeys infected while receiving isoniazid had developed no resistance, and in them the disease took its usual course once the drug was stopped.[4]

Chemoprophylaxis in Man

The Prevention of Infection (Primary Prophylaxis)

Although prophylaxis by isoniazid is most commonly indicated in tuberculin-positive subjects, its use has also been proposed for some susceptible but as yet uninfected people at special risk of infection. There is little known of the detailed consequences of such a course of action. It might be thought that, if a drug can cure a disease, it must surely be able to prevent it; but a number of examples can be given to show that this is often untrue. The fate of Schmidt's initially tuberculin-negative monkeys after the end of their year of isoniazid treatment shows that isoniazid had deferred but not prevented the serious consequences of their infection, and the work of Lelong and his associates (to be described later) shows that this course of events has its analogue in the human infant.

McDermott (16) has pointed out that a number of infectious diseases are less successfully treated soon after their inception than in the stage of florid disease. A particularly vivid example is provided by scrub typhus, since it has been shown (17) that, if chloramphenicol is started within a few hours after heavy exposure to the rickettsia, the disease is completely prevented as long as the administration of chloramphenicol is continued. Once the drug is stopped, however, the disease at once develops, even after forty days of prevention by the drug; this,

[4] Schmidt has recently pointed out that some of these animals may have received only very short periods of isoniazid *after* infection because both exposure to infection and the administration of isoniazid were stopped on the same day (15*b*).

despite the fact that only thirty-six to forty-eight hours' treatment with chloramphenicol is necessary for the cure of clinically apparent scrub typhus. These examples raise the possibility that isoniazid may merely postpone rather than prevent the course of events resulting from tuberculous infection in some subjects. This itself may, of course, be a valuable function of the drug, since the establishment of a primary complex at the age of, say, three years is much less dangerous than at the age of, say, one year.

In practice, there seems to be no reason for the widespread use of isoniazid in tuberculin-negative people, since the drug would have to be given to very large numbers of people in order to benefit a few at risk and because isoniazid would have to be given for an indefinite period. There are a few situations, however, in which isoniazid could reasonably be given to susceptible subjects, as yet either uninfected or not infected for a long enough period to have developed skin allergy, when such people are exposed to an unavoidably high risk of infection. This is most likely to happen when a child is born into an infectious environment and its separation from the source of infection is for some reason impossible.

Lelong and co-workers (18) treated four infants who had been in contact with tuberculous parents for various periods of time. Two of them remained tuberculin negative so that it was uncertain whether they had ever become infected. One was separated from a tuberculous mother at birth and given isoniazid from the eighteenth to the forty-first day of life. At the age of six weeks and at 2½ months the tuberculin reaction was negative, but at three months it became positive. Conversion had occurred six weeks after the end of treatment, but the child remained well with a normal chest roentgenogram. The fourth child provided a particularly vivid illustration of what might be expected of isoniazid prophylaxis in a small infant exposed to infection. The child was born to a tuberculous mother who, nine days after confinement, had a relapse, with sputum positive for tubercle bacilli. Contact between the mother and child was continuous from birth to the thirty-fifth day when the child was admitted to the hospital. The child had been breast-fed for the first nine days of life, and after this the mother gave him food without taking special precautions until the child was admitted to the hospital. Isoniazid and PAS were given to the child daily for three months, ending at the age of five months. Numerous tuberculin tests were given and the reactions were negative during treatment. Six weeks after the drugs were stopped and five and a half months after contact with the mother had been broken, the tuberculin reaction became positive for the first time. At the same time the child developed a fever and a small tuberculous focus at the right lung base accompanied by a pleural effusion. Tubercle bacilli were grown from the pleural fluid. Treatment was started again, and at thirteen months of age the child was well.

In this infant, chemotherapy seems to have prolonged the incubation period of the disease but not prevented its development, although later the same drugs effectively suppressed the established disease. The behavior of this infant's disease is clearly analogous to that of Schmidt's rhesus monkeys; and the risk of an infection, suppressed but not abolished by the drug, progressing to serious disease after the end of the period of treatment must be considered. For this reason, if isoniazid is given to tuberculin-negative infants at special risk, it should be continued for two to three years, until the risks of serious disease have declined appreciably.

Tuberculin-negative adolescents in contact with an infectious patient in the home and nurses working for a short period in a tuberculosis ward might also be given isoniazid during the period of special risk. In this group, a course of isoniazid might be a reasonable alternative to the more usual procedure of BCG vaccination, when the period of contact with infection is known and short and when the risk arises in a population with a generally low prevalence of infection.

In dealing with all of the foregoing examples of tuberculin-negative groups at special risk, the clinician is faced with a difficult choice between the benefits of immediate protection afforded by isoniazid and the disadvantage that the development or maintenance of immunity may be impaired by its use. The introduction of an isoniazid-resistant strain of BCG for vaccination, as suggested by Canetti, would afford a possible solution to this dilemma. Experiments on guinea pigs by Bretey and Canetti (19) indicated that isoniazid-resistant BCG was more effective than standard BCG as an immunizing agent. Other workers (20) found no striking difference between the immunizing capacity of an isoniazid-resistant

BCG and that of the parent strain. Another possible solution would be the development of an effective nonliving vaccine (21), the action of which would not be impaired by concomitant administration of antituberculous drugs.

The Prevention of Disease in Previously Infected Subjects (Secondary Prophylaxis)

General considerations: There is strong evidence (22, 23) that much serious disseminated tuberculosis occurs within a year or so of infection. For this reason it seems wise to give isoniazid for a period to any recent converter. The date of tuberculin conversion is, however, often hard to establish; and it might be argued that isoniazid should therefore be given to all tuberculin-positive people, since some of them are recent converters and at risk of developing serious disease. This course of action cannot be recommended because of our ignorance of its effects on the patients' subsequent immunity and on their liability to harbor drug-resistant bacilli.

In discussing the experimental work on chemoprophylaxis, it was pointed out that the period during which immunity continues to increase after infection is unknown; the likelihood of isoniazid's impairing the development of this immunity is therefore also unknown. This risk cannot be dismissed as theoretic, since there is evidence that allergy to tuberculin can diminish after isoniazid prophylaxis in children. It is at least possible, therefore, that the degree of immunity possessed by these children may also have diminished. Robinson, Meyer, and Middlebrook (24) reported that four infants, aged seven to twenty-four months, found on contact examination to have normal roentgenograms of the chest but reactions positive to 1:10,000 (Mantoux) reverted to negative reactions at 1:100 after six to seven months' treatment with isoniazid in a dose of 8 mg. per kg. per day. Two infants with positive skin reactions and normal chest roentgenograms remained positive, as did seven infants who had positive skin reactions and abnormal chest roentgenograms.

Choremis and associates (25) reported that three of eight children, allergic as a result of BCG vaccination, reverted to negative skin reactions after one month of treatment with isoniazid. On the other hand, in the U. S. Public Health Service study of isoniazid in primary tuberculosis, the small decrease in tuberculin sensitivity of children receiving isoniazid for a year was no greater than the decrease by natural loss of sensitivity in the control group.

Whether or not isoniazid affects a person's subsequent immunity to tuberculosis may depend largely on whether the bacilli are active or quiescent at the time the drug is given. It is possible that the risk both of lessening immunity and of developing drug resistance is greatest in those people for whom chemoprophylaxis is most effective, that is, those in the early stages of infection when the risks of serious disease are greatest. At a late stage after infection, when any remaining bacilli may be in a quiescent stage and therefore insusceptible to the drug, there will be little risk either of diminishing immunity or of encouraging the emergence of resistant strains. The role of isoniazid at this stage would be to abort rapidly any episodes of incipient relapse and to prevent bacterial multiplication resulting from exogenous reinfection.

It is obvious that, without actual trials in man, we are able to answer few of the important questions about the effects of isoniazid prophylaxis. The difficulty of predicting, even in a speculative way, the likely effects of isoniazid on immunity and on the chances of bacterial resistance derives partly from the crudity of our clinical assessments in tuberculosis. This discussion is restricted to prophylaxis in people without obvious evidence of disease; but a tuberculin-negative patient may already be infected and a tuberculin-positive patient with no clinical or roentgenographic evidence of disease may harbor tuberculous lesions of very variable age, extent, and activity.

It is those unknown factors which will determine the need of isoniazid prophylaxis, the likelihood of its success, and its risk. For example, a child with no obvious roentgenographic abnormality might be harboring a mediastinal lymph node on the point of rupture into a bronchus. Once rupture occurred, the risk of isoniazid resistance in this child would be as high as in the patient with obvious cavitary pulmonary tuberculosis. In practice, it is impossible to give streptomycin or PAS as prophylactic agents on any appreciable scale, and a companion drug for isoniazid suitable for widespread use is badly needed. However, in particular circumstances it may be considered reasonable to add PAS to the isoniazid; for example, small children are found to tolerate PAS extremely well, and the two drugs can be given together at least initially.

Until more facts about the general effects of chemoprophylaxis in man are available, its use probably should be confined to special groups known to have a greater than usual risk of developing tuberculous disease. Evidence about the effects of isoniazid prophylaxis in some of these special groups is now beginning to accumulate.

Observations in man: The most clear-cut indication for chemoprophylaxis is the discovery of a tuberculin-positive infant, since infancy is the most hazardous period of life in respect of the risks of tuberculous disease after infection and since the infant is also of necessity a more-or-less recent converter. An important body of evidence about the effects of isoniazid in asymptomatic primary tuberculosis in infancy and childhood is now accumulating as a result of the U. S. Public Health Service trial, of which a preliminary report has been published (26). The trial is a carefully designed control study, in which half the children receive 4 to 6 mg. of isoniazid per kg. daily for a year, and the other half receive an identical placebo. Any child less than three years of age with a skin reaction to 5 TU was included, as were children more than three years old who were reactors to 5 TU and who also had roentgenographic evidence of primary tuberculosis. Later in the trial, children over three years with evidence of recent tuberculin conversion were also included. Two thousand seven hundred and fifty children were involved in the trial, and the test and control groups proved to have very similar characteristics.

During the twelve months of treatment, 5 children in the isoniazid group and 26 children in the placebo group developed extrapulmonary complications of primary tuberculosis, a difference clearly significant on statistical analysis. The five complications in the isoniazid group included two cases of miliary tuberculosis which were both of doubtful significance, one because the child had taken the tablets for a short time only, and one because of an uncertain diagnosis. Adverse pulmonary changes were seen in the chest roentgenograms of 21 children in the isoniazid group and of 34 in the placebo group. This difference is not statistically significant. Most of these changes were interpreted as atelectases resulting from endobronchial obstruction. Tuberculous complications also developed in 6 other children judged, on review, to have had them in less severe form on entering the trial; 5 of these children were on the placebo and only one on isoniazid. The evidence in the preliminary report is insufficient to show the later course of these children after the end of their year of treatment, but at the time of writing there was no reason to think that isoniazid merely postponed complications. The subsequent follow-up study of these children will be of the greatest interest and importance.

A trial of chemoprophylaxis in children has also been conducted in France by Debré who has discussed in detail (27) the indications for isoniazid. The results of the main trial are not yet available, but Debré quotes the findings in a preventorium near Paris in which 1,062 children with primary tuberculosis were treated without drugs in the period 1948–1955. Pleurisy developed in 44, miliary tuberculosis in one, meningitis in 3, and pulmonary tuberculosis in 13. In the same period, 600 children were given PAS or isoniazid—PAS. There was one case of pulmonary tuberculosis in this group, but none of the children developed pleurisy or miliary or meningeal tuberculosis. In Italy, Zorini (28, 29) is an enthusiastic advocate of isoniazid prophylaxis, but differs from most clinicians in that he gives the drug to both tuberculin-negative and tuberculin-positive family contacts.

Chemoprophylaxis has also been suggested on theoretic grounds in certain tuberculin-positive subjects in the older age groups, although no factual evidence has yet been amassed about its effects in these people. First, evidence of recent tuberculin conversion at any age provides a reasonable indication for chemoprophylaxis, since the risks of serious disease are greatest soon after infection in older children and in adults as well as in infants. Evidence of recent conversion is given either by a reliable sequence of tuberculin tests showing the change from negative to positive, or by the recording of a single strongly positive test. A reaction of more than 15 mm. of induration to 3 TU does denote a probability of recent infection and a definite risk of disease (30).

Second, the general use of isoniazid in tuberculin-positive people might be considered following surveys in population in which their prevalence is low. As Mitchison (31) points out, it would still be important to screen for active disease, but as the group became smaller so would the administrative problem. He considers that it may be by this sort of approach that the final elimination of tuberculosis in a country

with an efficient public health service could be envisaged. The diminishing incidence of infection in many countries is quickly making this method of control a practical possibility.

Other indications for chemoprophylaxis: The discussion has so far been confined to the use of isoniazid to prevent tuberculous disease in otherwise healthy people. A number of nontuberculous diseases are associated with a higher than normal risk of tuberculosis, and the use of isoniazid has been suggested as a part of their management. Of these the most obvious example is the added risk of tuberculosis in any patient receiving steroid hormones in appreciable doses for a long period. There is a strong case for isoniazid administration in patients with quiescent tuberculosis who are receiving steroids. It is doubtful, however, whether, in view of our ignorance of the effects of isoniazid on immunity, the drug should be given to those who have no roentgenographic abnormality or other evidence of tuberculosis. Patients receiving steroids and those in the other categories mentioned are by the nature of their illness under constant medical care, and this should include roentgenography of the chest at regular intervals. A special problem, however, exists in patients with sarcoidosis who are receiving treatment with steroid hormones. The incidence of frank tuberculosis developing after sarcoidosis varies a good deal from region to region and with the clinical types of sarcoidosis observed. These differences are reflected in variations of treatment. The use of antituberculous drugs in sarcoidosis has become much less popular in recent years, but many physicians still add chemotherapy to the steroids when the lung changes of sarcoidosis are of considerable severity. One recent authority (32) takes the view that antituberculous drugs have no place in the treatment of sarcoidosis.

Other patients at special risk are those with pneumoconiosis and those who have had gastrectomy for peptic ulcer. In patients with pneumoconiosis, a positive tuberculin skin reaction might well be a reasonable indication for chemoprophylaxis, especially if the prevalence of infection in the area concerned is relatively low, since detection of small roentgenographic changes associated with the onset of tuberculosis may be difficult in these patients.

One uncommon but possible indication for chemoprophylaxis in either tuberculin-positive or tuberculin-negative subjects is an accidental laboratory inoculation with tubercle bacilli. Here, too, the possibility that preventive treatment soon after infection may be ineffective and merely prolong the incubation period is a real one, and the decision whether or not to give chemotherapy must be an individual one taking account of the particular circumstances of each case.

If it is doubtful whether tubercle bacilli have actually been injected, it would be reasonable to withhold chemotherapy but to observe carefully and at frequent intervals the site of possible inoculation, the local lymph nodes, and the general condition of the patient. If it seems certain that an inoculum has been introduced, it would be proper to give a course of chemotherapy for at least a year and to follow this by clinical examination at frequent intervals for at least a year after the chemotherapy has been stopped. In a laboratory accident the infecting dose may be a large one and for this reason, if drug treatment is given, combined chemotherapy rather than isoniazid alone should be administered (31). Tuberculin tests are unlikely to be of value because persons involved in laboratory work on tuberculosis should already be tuberculin positive, either by natural infection or by BCG vaccination.

Discussion

It is clear that few of the questions posed at the beginning of this review about the effects of isoniazid prophylaxis have yet been answered by trials in man. The U. S. Public Health Service trial has shown that in children, as in experimental animals, isoniazid is highly effective in preventing serious tuberculous disease during the period of its administration. The later course of patients who have received chemoprophylaxis is not yet known in any detail, nor is it known whether occasional episodes of serious drug toxicity will arise in large populations which are given isoniazid, and what will be their frequency. Until more facts about the effects of chemoprophylaxis in man are available, the precise indications for its use will remain difficult to formulate, since the strength of the evidence for its employment varies greatly from case to case.

Ferebee (33) has outlined the program of trials now being carried out by the U. S. Public Health Service, which should, when completed, answer many of the important outstanding questions about isoniazid prophylaxis in man. Both

tuberculin-positive and tuberculin-negative contacts are included in a double-blind trial in household contacts of newly discovered cases. The initially tuberculin-negative subjects are undergoing further roentgenography and tuberculin testing six months after the one-year course of isoniazid has been completed. In this way it will be possible to find out if isoniazid is actually preventing infection or merely supressing it. It is hoped also by such trials to gain further information on other points, such as the incidence of drug toxicity and the characteristics of those tuberculin-positive reactors who are at greatest risk of developing disease. In the present state of our knowledge, the indications for the chemoprophylaxis of tuberculosis can be summarized as follows:

Definite indications:

1. Tuberculin-positive children less than three years of age.

2. Tuberculin-negative children less than three years of age who are exposed to continued and unavoidable risk. In these and in other tuberculin-negative subjects in whom prophylaxis is indicated, the development of an isoniazid-resistant strain of BCG or an effective nonliving vaccine would enable the advantages of vaccination to be combined with those of prophylaxis. In groups 1 and 2 the age given, three years, is to some extent an arbitrary one. For certain ethnic groups of great susceptibility, a serious risk may exist up to the age of five years or more, while in other populations the risk of disease after infection may already be quite low by the age of two years.

3. Tuberculin-positive people of any age whose tuberculin conversion is known to be a recent event. This is certain if a reliably performed sequence of tests has shown the conversion; it may perhaps be assumed if a single test shows a strongly positive reaction.

Possible indications:

1. Short-term contacts of tuberculous patients, especially nurses employed for a short time in tuberculosis wards. In these people, as in the tuberculin-negative infants already discussed, the possibility of deferring rather than preventing the sequence of events resulting from infection must be reckoned with. Here, too, a method of combining vaccination with prophylaxis would be an important advance.

2. Patients on long-term treatment with steroids. Zorini (29) would add to this group patients with pneumoconiosis, patients who have had gastrectomies, and those who have suffered severe viral infections. Most authorities would consider these indications too widespread in people without evidence of tuberculous disease. In those, however, with evidence of healed tuberculosis, indications such as these should be seriously considered. For example, a period of isoniazid prophylaxis might not be an unreasonable precaution in a patient with past serious tuberculosis who suffers a severe attack of acute respiratory disease such as Asian influenza.

3. Accidental infections with tuberculosis. If chemotherapy is given at all, at least two drugs should be administered, since the infecting dose may be a large one.

All of the indications discussed have been concerned with special groups, and there is as yet little justification for considering the use of isoniazid on a larger scale. The disadvantages of its use in tuberculin-negative populations are that it would benefit so few of the people to whom it was given and that it would have to be given for an indefinite period. The main factor mitigating against its widespread use in the tuberculin-positive subject is our ignorance of the effects of chemoprophylaxis on immunity and on the chances of drug resistance. These unknown factors are certainly important in a population with a high prevalence of tuberculosis and with many sources of infection, but they may not be very important in an area of low prevalence. For this reason chemoprophylaxis could perhaps be used more widely in tuberculin-positive subjects in areas where they constitute only a small proportion of the population.

Finally, it must be emphasized that this discussion of chemoprophylaxis has been arbitrarily restricted to those persons with no obvious evidence of tuberculous disease. But, in a sense, treatment of established tuberculous disease is often partly or entirely prophylactic in its aims. The treatment of primary tuberculosis in childhood, which has been reviewed recently by several writers (34, 35, 36), is directed at the prevention of complications rather than at the existing lung lesion. Chemotherapy in tuberculous pleural effusion has little, if any, effect on the course of the illness itself, but does vastly reduce the chances of subsequent pulmonary tuberculosis (37). In chronic pulmonary tuberculosis the prevention of relapse is as much a concern of treatment as is the immediate control of the infection.

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FATALITY RATIO FOR LUPUS VULGARIS CUTIS[1, 2]

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Introduction

Lupus vulgaris cutis is considered the most severe of the tuberculous skin diseases. In the present paper, its significance is appraised by the fatality ratio, a method devised to show whether the number of deaths among patients with lupus exceeds the number expected according to the mortality in the general population.

The fatality ratio is suggested as a more pertinent estimate of the severity of a disease than, for example, case-fatality rates, five-year survival percentages, or lethality. These methods demonstrate the number of patients dying from a disease, but do not elucidate whether this number is higher than the number of deaths otherwise observed in the general population. Such a comparison is provided by the fatality ratio, and this method is therefore considered to have a broad application in public health and clinical work.

Material and Method

Patients: During the years 1895–1954, a total of 3,902 patients with lupus vulgaris cutis (hereafter referred to as lupus) were admitted to the Finsen Institute, Copenhagen. These patients probably comprise more than 80 per cent of all cases diagnosed in Denmark during this period (1).

The fatality ratios were calculated on the basis of the patients who were known to have died within the period 1895–1954 or to have been alive at the end of 1954. Three thousand three hundred and thirteen patients were included, 980 males and 2,333 females. Thus, of the original 3,902 patients, 589 were excluded from the study: 465 who could not be identified in the municipal registers, 67 who emigrated from Denmark within the period, and 57 whose age at death could not be ascertained. The municipal register system, set up in 1924, comprises a complete listing of the local population, including removals by migration and death. It is therefore generally possible to trace most of the population of the country. The 465 patients who could not be identified in the registers, most of whom were admitted before 1924, were excluded as there was no other way to determine whether or not they were living or dead.

Fatality ratio: By the fatality ratio is understood the ratio between the observed and the expected numbers of deaths. The expected number is that which would have been found if the overall mortality rate of the patient-group was the same as that for the general population.

Observed number of deaths: The observed number of deaths could be counted directly from the records of the Finsen Institute. They have been distributed in five-year age groups according to the age of the patient at death. The deaths of 121 patients were stated to have occurred within a certain time period, which, however, in no instance exceeded ten years; for these patients the age at the middle of the given period was used.

Expected number of deaths: The expected number of deaths was calculated on the basis of mortality tables available in the Danish statistical tables and yearbooks.[3] The calculations were made as follows:

Let us say that in the decade 1895–1904 n patients aged a years[4] were admitted to the Finsen Institute. The proportion of these patients expected to be alive after $2\frac{1}{2}$ years was then calculated: in the mortality table for 1901–1905, the proportion of the population aged a alive after $2\frac{1}{2}$ years is shown by: $F_1 = \frac{l_a + 2\frac{1}{2}}{l_a}$, where l indicates the number of persons aged a. If the n patients aged a survived in the same way as the general population, there would be $n \times F_1 = n_1$ patients alive $2\frac{1}{2}$ years after admission, while $n - n_1$ patients would have died in the same period of time. Next, the number of n_1 patients aged $a + 2\frac{1}{2}$ years alive ten years later was calcu-

[1] From the Dermatological Department, Finsen Institute, Copenhagen, Denmark.

[2] This study was supported by grants from the King Christian X's Foundation and the Augustinus' Foundation, Denmark.

[3] A mortality table shows how 100,000 newborn infants would die if the mortality by age did not change with time. As mortality does change with time, new mortality tables are prepared every five years. The calculations are based on the mortality observed through the 5-year period. In the present study mortality tables for the periods: 1901–1905, 1906–1910, . . . 1951–1955 have been used (2, 3).

[4] The patients were grouped in 5-year age groups by age at admission; and a corresponds to the average age of each group, i.e., $2\frac{1}{2}$ years, $7\frac{1}{2}$ years, et cetera. The ages subsequently defined, $a + 2\frac{1}{2}$ years, $a + 12\frac{1}{2}$ years, et cetera, are therefore whole numbers.

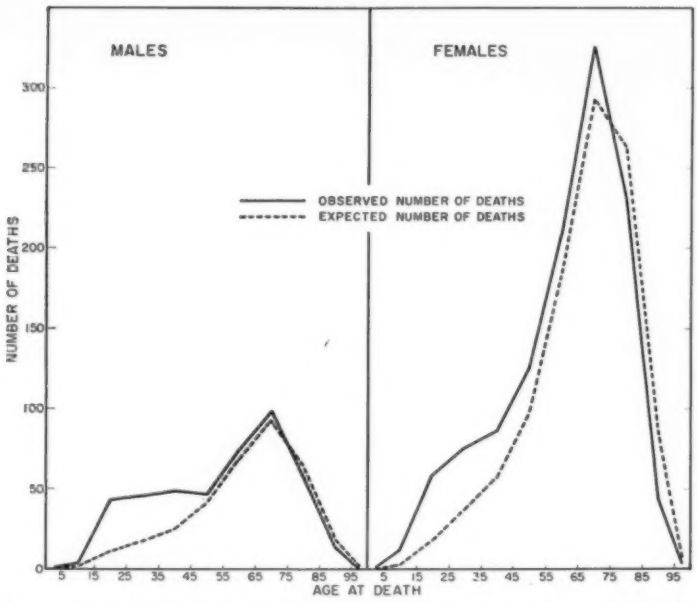

FIG. 1. Observed and expected number of deaths in lupus patients admitted 1895–1954, by age at death.

lated. The mortality table for 1906–1910 shows how large a proportion of the population aged $a + 2\frac{1}{2}$ years would be alive after 10 years: $F_2 = \frac{l_a + 12\frac{1}{2}}{l_a + 2\frac{1}{2}}$. If the n_1 patients survived as the general population, there would be $n_1 \times F_2 = n_2$ patients alive 10 years later, while $n_1 - n_2$ patients would have died in the same period. This brings the original group of n patients aged a up to a point of time $12\frac{1}{2}$ years removed from admission. By continuing in this way, the age group a was followed until January 1, 1955, the date of conclusion of the study.[5] The total number of deaths expected among the original n patients aged a could thereafter be found by simple addition.[6]

Similar calculations were made for patients in all age groups admitted in the decade 1895–1904. Where a (age) at admission was advanced, all patients would have died before January 1, 1955; this was in fact assumed for the 95-year age group.

Similar calculations were thereafter made for patients admitted in the decades 1905–1914, 1915–1924 . . . 1945–1954. From the total series of calculations it was possible to determine, for individual age groups and for the total patient material, the number of expected deaths and the ages at which they would have occurred.

RESULTS

Crude fatality ratio: Of the 980 male patients, 437 died within the period 1895–1954. It may be seen in figure 1 and table 1*a* that only a few died in childhood. At age 20 the number of deaths increased considerably, but thereafter remained at an almost constant level until the age of 50, when it rose again to a marked peak around 70 years. The expected number of deaths was 346, and is illustrated by the broken curve in figure 1. This curve rises slowly and steadily from childhood until the age of 50 and thereafter resembles the curve for the observed deaths. Up to the age of 70 the observed number of deaths is higher than the expected number, and thereafter it is a little lower. The ratio between the total numbers of observed and expected deaths, the summary fatality ratio, is 126 per cent. From childhood until 35 years of age the ratio varies about 300 per cent, after which it gradually falls to 70 per cent at age 90. This

[5] The calculations were made for the following intervals from admission: 0–$2\frac{1}{2}$ years, $2\frac{1}{2}$–$12\frac{1}{2}$ years, $12\frac{1}{2}$–$22\frac{1}{2}$ years, $22\frac{1}{2}$–$32\frac{1}{2}$ years, $32\frac{1}{2}$–$42\frac{1}{2}$ years, $42\frac{1}{2}$–$52\frac{1}{2}$ years, and $52\frac{1}{2}$–55 years.

[6] In tables 1–6 the numbers of deaths are given by larger age groups. They have been calculated by interpolation.

TABLE 1*a*

MALE PATIENTS: OBSERVED AND EXPECTED NUMBER OF DEATHS IN LUPUS PATIENTS ADMITTED 1895–1954, BY AGE AT ADMISSION AND AGE AT DEATH

Age at Admission (years)	Age at Death (years)											Total
	0–4	5–14	15–24	25–34	35–44	45–54	55–64	65–74	75–84	85–94	95+	
Number Observed												
0–4	1	1	1	—	—	—	—	•	•	•	•	3
5–14	•	3	18	2	6	7	3	1	•	•	•	40
15–24	•	•	25	29	21	6	18	20	3	•	•	122
25–34	•	•	•	15	13	7	12	11	3	—	•	61
35–44	•	•	•	•	9	15	15	17	14	4	—	74
45–54	•	•	•	•	•	12	17	20	19	2	—	70
55–64	•	•	•	•	•	•	10	16	8	2	—	36
65+	•	•	•	•	•	•	•	14	11	6	—	31
Total.....	1	4	44	46	49	47	75	99	58	14	—	437
Number Expected												
0–4	0.2	0.3	0.4	0.3	0.2	0.1	o.o	•	•	•	•	1.5
5–14	•	1.4	5.1	4.8	3.9	4.7	4.8	1.3	•	•	•	26.0
15–24	•	•	5.4	10.3	11.0	13.3	17.7	18.5	3.2	•	•	79.4
25–34	•	•	•	2.5	5.9	7.5	11.4	13.5	8.4	0.5	•	49.7
35–44	•	•	•	•	3.8	9.9	14.4	17.1	11.6	3.1	0.2	60.1
45–54	•	•	•	•	•	6.1	15.4	21.0	16.4	5.1	0.3	64.3
55–64	•	•	•	•	•	•	5.8	14.2	11.4	3.2	0.2	34.8
65+	•	•	•	•	•	•	•	7.7	14.9	7.2	0.6	30.4
Total.....	0.2	1.7	10.9	17.9	24.8	41.6	69.5	93.3	65.9	19.1	1.3	346.2

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• Category not applicable

TABLE 1*b*

FEMALE PATIENTS: OBSERVED AND EXPECTED NUMBER OF DEATHS IN LUPUS PATIENTS ADMITTED 1895–1954, BY AGE AT ADMISSION AND AGE AT DEATH

Age at Admission (years)	Age at Death (years)											Total
	0–4	5–14	15–24	25–34	35–44	45–54	55–64	65–74	75–84	85–94	95+	
Number Observed												
0–4	1	1	1	2	1	—	—	•	•	•	•	6
5–14	•	11	18	7	2	6	3	1	•	•	•	48
15–24	•	•	40	38	20	18	31	23	2	•	•	172
25–34	•	•	•	29	37	29	32	56	29	1	•	213
35–44	•	•	•	•	26	45	39	55	44	3	—	212
45–54	•	•	•	•	•	27	70	74	57	8	1	237
55–64	•	•	•	•	•	•	34	81	49	14	1	179
65+	•	•	•	•	•	•	•	36	49	18	1	104
Total.....	1	12	59	76	86	125	209	326	230	44	3	1,171
Number Expected												
0–4	0.3	0.6	0.7	0.6	0.5	0.2	o.o	•	•	•	•	2.9
5–14	•	2.1	7.4	7.5	6.4	6.4	5.7	1.4	•	•	•	36.9
15–24	•	•	9.9	21.2	21.3	24.4	30.7	32.1	6.3	•	•	145.9
25–34	•	•	•	8.3	19.8	24.9	36.7	47.1	36.2	3.3	•	176.3
35–44	•	•	•	•	9.8	24.7	39.0	54.6	46.2	15.4	1.1	190.8
45–54	•	•	•	•	•	16.6	47.0	72.0	64.8	21.1	1.5	223.0
55–64	•	•	•	•	•	•	23.8	61.5	60.3	22.1	1.7	169.4
65+	•	•	•	•	•	•	•	24.2	49.3	25.1	2.5	101.1
Total.....	0.3	2.7	18.0	37.6	57.8	97.2	182.9	292.9	263.1	87.0	6.8	1,046.3

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o.o Magnitude less than half unit employed
• Category not applicable

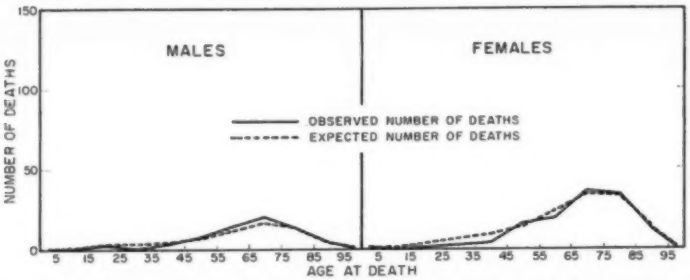

FIG. 2. Observed and expected number of deaths in lupus patients admitted 1895–1954 in whom lupus was the only tuberculous disease, by age at death.

"under-mortality" in older ages cannot be taken as a sign of increased resistance, e.g., as a result of treatment, but is a consequence of the "excess mortality" in the younger ages: patients who have died in youth cannot die in the older age groups.

Similar variations are observed among female patients. The rise in the observed number of deaths among adolescents and young adults, however, is less pronounced (figure 1 and table 1*b*). Of the 2,333 female patients, 1,171 died. The expected number of deaths was 1,046, and the summary fatality ratio was thus 112 per cent. From childhood until 35 years the fatality ratio varies about 250 per cent, and thereafter falls gradually to 50 per cent at the age of 90 years.

Summary fatality ratios by age at admission can also be computed from tables 1*a* and *b*. The ratios are high in the age groups 0–14 years and thereafter decrease steadily with increasing age, approaching 100 per cent. However, for all age groups the fatality ratios are high in the first years after admission.

Variation of crude fatality ratio with occurrence of other tuberculous disease: The form of the curve for observed deaths suggested that there was a group with a particularly high fatality ratio. It seemed reasonable to investigate whether this group comprised patients subject to other tuberculous disease as well as lupus.

The patients were, accordingly, classified in the following groups: (*a*) those with lupus as the only tuberculous disease; (*b*) those with a history of other forms of nonrespiratory tuberculosis, but not of respiratory tuberculosis; (*c*) those with a history of respiratory tuberculosis. In 80 to 85 per cent of these there was a history of nonrespiratory tuberculosis as well.[7]

The classification was based on the patient's complete history and not on status at admission alone. As it is known that lupus patients often die from other tuberculous diseases, 217 patients for whom the cause of death was unknown were excluded from this investigation. A further 133 were excluded because the temporal relation of lupus to other tuberculous disease was unknown.

The age distribution of patients at admission was not quite similar for the three groups, but the differences are considered too small to account for the differences in the summary fatality ratios observed.

Lupus only: Two hundred and one male and 430 female patients belonged to the lupus group. There was no essential difference between observed and expected deaths (figure 2, tables 2*a* and *b*), i.e., the mortality rate was the same for these patients as for the general population.

Lupus plus nonrespiratory tuberculosis: The group with lupus plus nonrespiratory tuberculosis comprised 512 male and 1,230 female patients. The mortality rate was virtually the same for these patients as for the general population (figure 3, tables 3*a* and *b*). In adolescence and young adulthood, the fatality ratios are raised, 200 per cent for males and 150 per cent for females; but this explains only part of the increased ratios found for the total patient material in these age groups.

Lupus plus respiratory tuberculosis: One

[7] For brevity, the three groups are designated hereafter as "lupus only," "lupus plus nonrespiratory tuberculosis," and "lupus plus respiratory tuberculosis."

TABLE 2*a*

MALE PATIENTS: OBSERVED AND EXPECTED NUMBER OF DEATHS IN LUPUS PATIENTS ADMITTED 1895–1954 IN WHOM LUPUS WAS THE ONLY TUBERCULOUS DISEASE, BY AGE AT ADMISSION AND AGE AT DEATH

Age at Admission (years)	Age at Death (years)											Total
	0–4	5–14	15–24	25–34	35–44	45–54	55–64	65–74	75–84	85–94	95+	
Number Observed												
0–4	—	—	—	—	—	—	•	•	•	•	•	—
5–14	•	—	—	—	1	2	2	—	•	•	•	5
15–24	•	•	2	—	2	1	2	6	—	•	•	13
25–34	•	•	•	—	—	—	1	2	2	•	•	5
35–44	•	•	•	•	—	3	5	3	1	—	•	12
45–54	•	•	•	•	•	1	3	1	6	—	—	11
55–64	•	•	•	•	•	•	1	3	1	1	•	6
65+	•	•	•	•	•	•	•	5	3	3	—	11
Total.....	—	—	2	—	3	7	14	20	13	4	—	63
Number Expected												
0–4	0.1	0.1	0.1	0.1	0.1	o.o	•	•	•	•	•	0.5
5–14	•	0.4	1.4	1.1	1.0	1.2	0.7	0.1	•	•	•	5.9
15–24	•	•	0.8	1.5	1.4	1.8	2.6	2.7	0.4	•	•	11.2
25–34	•	•	•	0.4	0.9	1.2	1.4	1.3	0.7	•	•	5.9
35–44	•	•	•	•	0.6	1.7	2.4	2.2	1.2	0.2	•	8.3
45–54	•	•	•	•	•	1.2	3.0	3.6	2.7	0.8	0.1	11.4
55–64	•	•	•	•	•	•	1.3	3.5	2.5	0.4	•	7.7
65+	•	•	•	•	•	•	•	2.7	5.8	2.4	0.1	11.0
Total.....	0.1	0.5	2.3	3.1	4.0	7.1	11.4	16.1	13.3	3.8	0.2	61.9

— Magnitude nil o.o Magnitude less than half unit employed • Category not applicable

TABLE 2*b*

FEMALE PATIENTS: OBSERVED AND EXPECTED NUMBER OF DEATHS IN LUPUS PATIENTS ADMITTED 1895–1954 IN WHOM LUPUS WAS THE ONLY TUBERCULOUS DISEASE, BY AGE AT ADMISSION AND AGE AT DEATH

Age at Admission (years)	Age at Death (years)											Total
	0–4	5–14	15–24	25–34	35–44	45–54	55–64	65–74	75–84	85–94	95+	
Number Observed												
0–4	1	—	—	1	—	—	—	•	•	•	•	2
5–14	•	—	—	—	1	1	1	1	•	•	•	4
15–24	•	•	1	1	1	2	2	3	—	•	•	10
25–34	•	•	•	—	—	4	4	5	2	1	•	16
35–44	•	•	•	•	2	6	3	4	5	1	•	21
45–54	•	•	•	•	•	3	5	11	6	1	—	26
55–64	•	•	•	•	•	•	4	6	11	3	—	24
65+	•	•	•	•	•	•	•	6	10	6	—	22
Total.....	1	—	1	2	4	16	19	36	34	12	—	125
Number Expected												
0–4	0.1	0.3	0.3	0.3	0.2	0.1	o.o	•	•	•	•	1.3
5–14	•	0.6	1.8	1.7	1.5	1.5	1.0	0.2	•	•	•	8.3
15–24	•	•	1.6	3.2	3.2	3.3	3.4	3.4	0.6	•	•	18.7
25–34	•	•	•	1.1	2.5	2.9	3.7	3.8	2.4	0.2	•	16.6
35–44	•	•	•	•	1.5	3.4	4.7	5.8	4.6	1.7	•	21.7
45–54	•	•	•	•	•	2.6	6.6	9.1	7.3	2.3	0.2	28.1
55–64	•	•	•	•	•	•	3.6	9.1	8.8	3.0	0.2	24.7
65+	•	•	•	•	•	•	•	4.0	9.8	5.4	0.5	19.7
Total.....	0.1	0.9	3.7	6.3	8.9	13.8	23.0	35.4	33.5	12.6	0.9	139.1

— Magnitude nil o.o Magnitude less than half unit employed • Category not applicable

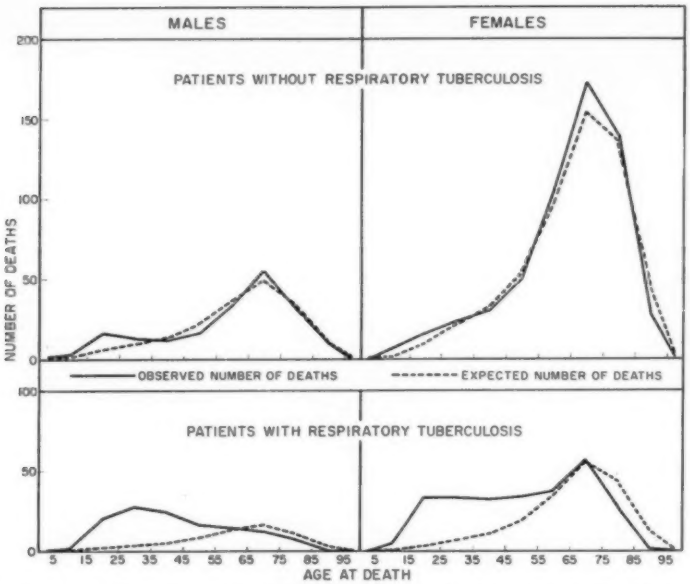

FIG. 3. Observed and expected number of deaths in lupus patients admitted 1895–1954 in whom other forms of tuberculous disease had occurred, by age at death.

hundred and eighty-seven male and 403 female patients belonged to the group with lupus plus respiratory tuberculosis. In all age groups except the oldest, the observed number of deaths exceeded the expected (figure 3 and tables 4*a* and *b*). This was particularly pronounced in the young in whom the fatality ratio for male patients was about 850 per cent and for female about 700 per cent. It is, thus, chiefly this group of patients that accounts for the rise in deaths from childhood to 35 years shown in figure 1.

Variation of fatality ratio with length of observation period: In this section the observed and expected numbers of deaths are illustrated as cumulative distribution functions.[8] In this way the total number of deaths occurring since admission can be shown at any point in time. The charts and tables can thus be used as survival tables as well. They show, for example, how many of the patients attained a certain age and how many were alive at different intervals after admission.

The observation of a group ends when all of the patients have died. The fatality ratio at this point in time, designated the terminal fatality ratio, is one expression of the severity of a disease. Suppose, for instance, that a disease of childhood caused all of 100 afflicted children to die in the course of 10 years. During this period only a few deaths would be expected, say 4. This would give a terminal fatality ratio of 100 ÷ 4, or 2,500 per cent.

The course of the disease in different groups of patients is shown in figures 4*a* and *b* and tables 5*a* and *b*. During the first years after admission, a much greater number died than was expected. The difference was relatively more pronounced among those admitted as children and young adults. After continued observation, the observed and the expected numbers of deaths rise, but the difference between them becomes relatively small, and in some instances even disappears. Thus for all age groups the fatality ratio tends toward 100 per cent. After a sufficiently long observation period, all of the patients have died. For these patients the terminal fatality ratio is 100 per cent.

Only a few age groups could be followed long enough for all or nearly all of the patients to

[8] The observed and the expected numbers of deaths have been converted to correspond to the admission of 100 patients in each group.

TABLE 3*a*

MALE PATIENTS: OBSERVED AND EXPECTED NUMBER OF DEATHS IN LUPUS PATIENTS ADMITTED 1895–1954 IN WHOM OTHER FORMS OF NON-RESPIRATORY TUBERCULOSIS, BUT NOT RESPIRATORY TUBERCULOSIS, HAD OCCURRED, BY AGE AT ADMISSION AND AGE AT DEATH

Age at Admission (years)	Age at Death (years)											Total
	0–4	5–14	15–24	25–34	35–44	45–54	55–64	65–74	75–84	85–94	95+	
Number Observed												
0–4	1	—	1	—	—	—	—	•	•	•	•	2
5–14	•	3	7	—	1	1	—	—	•	•	•	12
15–24	•	•	8	9	7	2	8	10	1	•	•	45
25–34	•	•	•	4	1	5	9	9	1	—	•	29
35–44	•	•	•	•	3	4	4	10	10	4	•	35
45–54	•	•	•	•	•	4	6	13	9	2	—	34
55–64	•	•	•	•	•	•	6	6	5	1	—	18
65+	•	•	•	•	•	•	•	7	5	3	—	15
Total.....	1	3	16	13	12	16	33	55	31	10	—	190
Number Expected												
0–4	0.1	0.2	0.2	0.2	0.0	0.1	0.0	•	•	•	•	0.8
5–14	•	0.7	2.2	2.2	1.8	1.9	2.1	0.6	•	•	•	11.5
15–24	•	•	2.9	5.8	6.2	7.5	9.4	9.8	2.1	•	•	43.7
25–34	•	•	•	1.4	3.3	4.2	6.7	7.8	5.0	0.3	•	28.7
35–44	•	•	•	•	2.0	5.3	7.4	9.4	6.7	1.8	•	32.6
45–54	•	•	•	•	•	2.9	7.6	10.6	8.0	2.4	0.2	31.7
55–64	•	•	•	•	•	•	3.0	6.9	6.2	2.1	0.1	18.3
65+	•	•	•	•	•	•	•	3.4	5.9	3.5	0.5	13.3
Total.....	0.1	0.9	5.3	9.6	13.3	21.9	36.2	48.5	33.9	10.1	0.8	180.6

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TABLE 3*b*

FEMALE PATIENTS: OBSERVED AND EXPECTED NUMBER OF DEATHS IN LUPUS PATIENTS ADMITTED 1895–1954 IN WHOM OTHER FORMS OF NON-RESPIRATORY TUBERCULOSIS, BUT NOT RESPIRATORY TUBERCULOSIS, HAD OCCURRED, BY AGE AT ADMISSION AND AGE AT DEATH

Age at Admission (years)	Age at Death (years)											Total
	0–4	5–14	15–24	25–34	35–44	45–54	55–64	65–74	75–84	85–94	95+	
Number Observed												
0–4	—	1	—	1	1	—	—	•	•	•	•	3
5–14	•	6	5	5	—	4	1	—	•	•	•	21
15–24	•	•	11	11	8	6	18	12	1	•	•	67
25–34	•	•	•	6	10	14	20	32	23	—	•	105
35–44	•	•	•	•	11	16	15	40	28	2	•	112
45–54	•	•	•	•	•	10	34	36	33	5	—	118
55–64	•	•	•	•	•	•	17	35	24	11	1	88
65+	•	•	•	•	•	•	•	18	30	10	1	59
Total.....	—	7	16	23	30	50	105	173	139	28	2	573
Number Expected												
0–4	0.1	0.3	0.3	0.3	0.2	0.1	0.0	•	•	•	•	1.3
5–14	•	1.0	3.8	3.9	3.3	3.5	3.1	0.8	•	•	•	19.4
15–24	•	•	5.4	11.8	11.7	13.3	16.8	17.3	3.6	•	•	79.9
25–34	•	•	•	4.8	10.8	13.6	20.4	25.0	18.7	1.6	•	94.9
35–44	•	•	•	•	5.6	13.8	22.0	30.1	24.6	8.5	•	104.6
45–54	•	•	•	•	•	7.8	23.8	36.1	31.3	8.9	0.6	108.5
55–64	•	•	•	•	•	•	11.5	30.8	30.0	10.8	0.9	84.0
65+	•	•	•	•	•	•	•	14.0	28.1	14.2	1.7	58.0
Total.....	0.1	1.3	9.5	20.8	31.6	52.1	97.6	154.1	136.3	44.0	3.2	550.6

— Magnitude nil 0.0 Magnitude less than half unit employed • Category not applicable

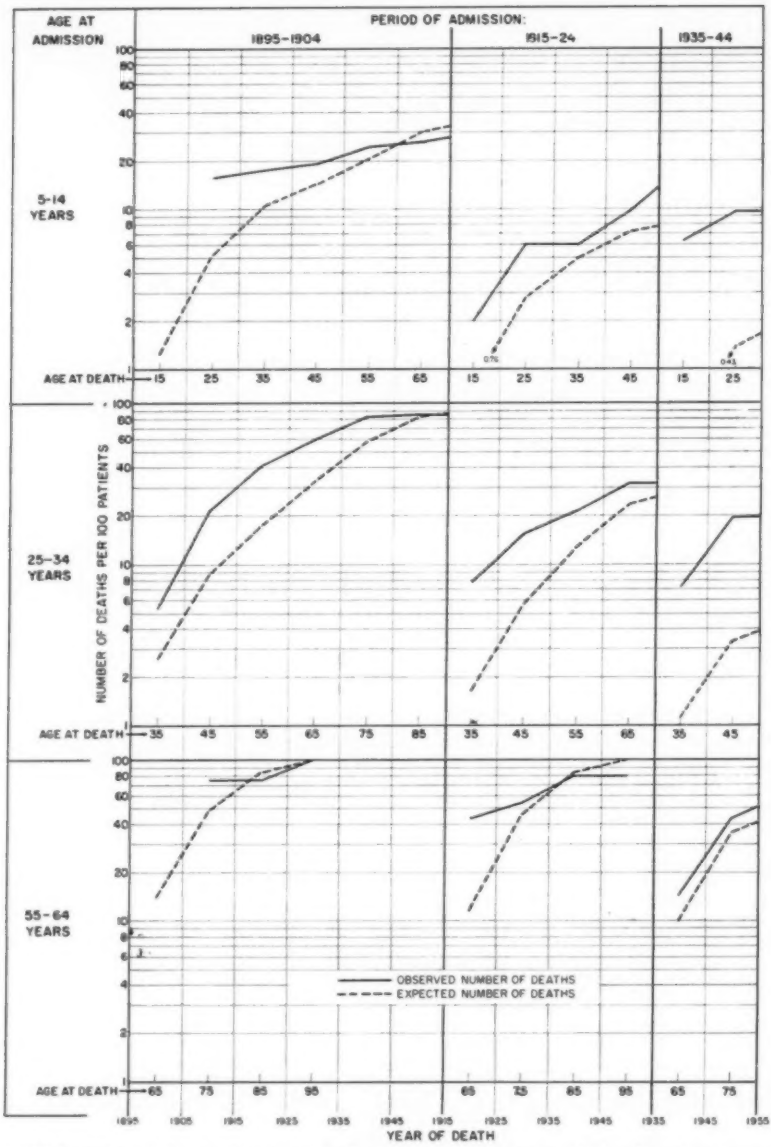

FIG. 4*a*. Male patients: observed and expected number of deaths as cumulative function in percentage, n lupus patients admitted, by decade of admission, age at admission, and age at death.

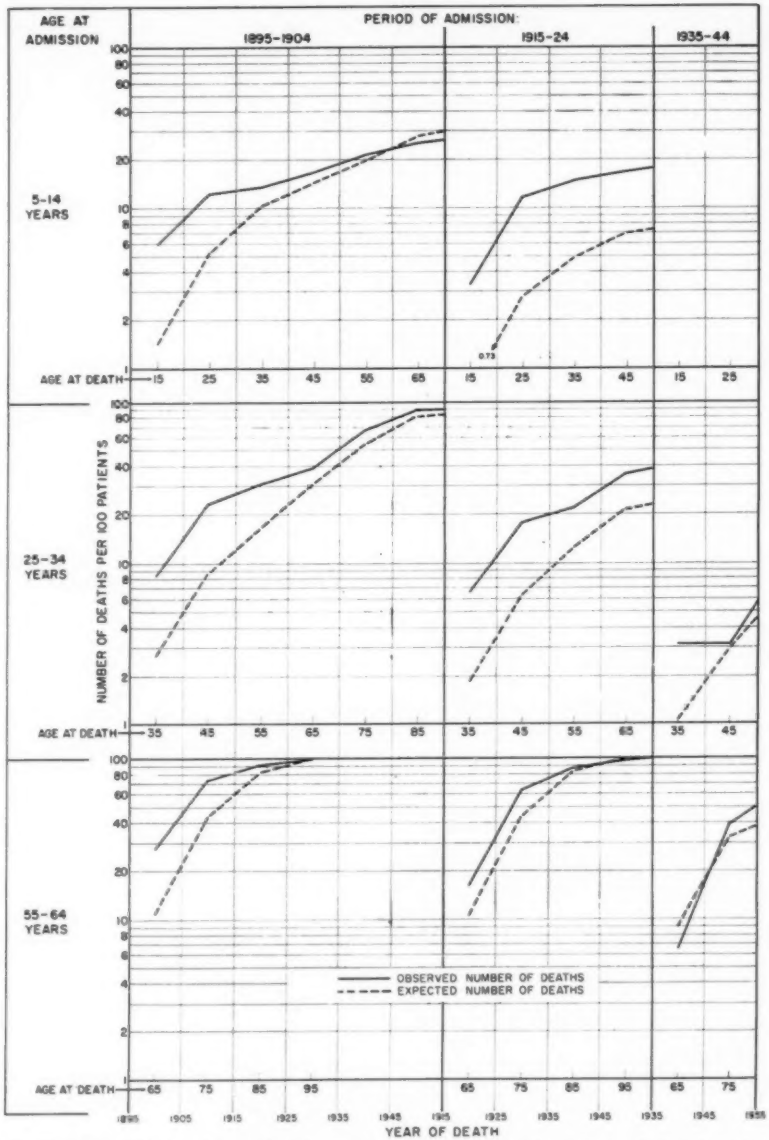

FIG. 4*b*. Female patients: observed and expected number of deaths, as cumulative function in percentage, in lupus patients admitted, by decade of admission, age at admission, and age at death.

TABLE 4*a*

MALE PATIENTS: OBSERVED AND EXPECTED NUMBER OF DEATHS IN LUPUS PATIENTS ADMITTED 1895–1954 IN WHOM RESPIRATORY TUBERCULOSIS HAD OCCURRED, BY AGE AT ADMISSION AND AGE AT DEATH

Age at Admission (years)	Age at Death (years)											Total
	0–4	5–14	15–24	25–34	35–44	45–54	55–64	65–74	75–84	85–94	95+	
Number Observed												
0–4	—	1	—	—	•	•	•	•	•	•	•	1
5–14	•	—	9	1	3	4	—	—	•	•	•	17
15–24	•	•	12	17	8	2	5	2	1	•	•	47
25–34	•	•	•	10	11	1	1	—	—	—	•	23
35–44	•	•	•	•	3	6	2	3	3	—	•	17
45–54	•	•	•	•	•	4	5	3	1	—	—	13
55–64	•	•	•	•	•	•	2	4	1	—	—	7
65+	•	•	•	•	•	•	•	1	2	—	—	3
Total.....	—	1	21	28	25	17	15	13	8	—	—	128
Number Expected												
0–4	0.0	0.0	0.0	0.0	•	•	•	•	•	•	•	0.0
5–14	•	0.1	0.9	1.0	0.8	1.1	1.4	0.4	•	•	•	5.7
15–24	•	•	1.1	2.1	2.3	2.7	3.3	3.3	0.4	•	•	15.2
25–34	•	•	•	0.6	1.4	1.8	2.9	3.7	2.1	0.2	•	12.7
35–44	•	•	•	•	0.8	1.9	2.9	3.1	1.7	0.5	•	10.9
45–54	•	•	•	•	•	1.1	2.4	3.1	2.6	0.7	0.0	9.9
55–64	•	•	•	•	•	•	1.3	2.2	1.7	0.5	0.0	5.7
65+	•	•	•	•	•	•	•	0.9	2.3	0.8	0.0	4.0
Total.....	0.0	0.1	2.0	3.7	5.3	8.6	14.2	16.7	10.8	2.7	0.0	64.1

— Magnitude nil 0.0 Magnitude less than half unit employed • Category not applicable

TABLE 4*b*

FEMALE PATIENTS: OBSERVED AND EXPECTED NUMBER OF DEATHS IN LUPUS PATIENTS ADMITTED 1895–1954 IN WHOM RESPIRATORY TUBERCULOSIS HAD OCCURRED, BY AGE AT ADMISSION AND AGE AT DEATH

Age at Admission (years)	Age at Death (years)											Total
	0–4	5–14	15–24	25–34	35–44	45–54	55–64	65–74	75–84	85–94	95+	
Number Observed												
0–4	—	—	1	—	—	—	•	•	•	•	•	1
5–14	•	5	10	2	—	—	—	—	•	•	•	17
15–24	•	•	23	16	7	4	4	7	1	•	•	62
25–34	•	•	•	16	18	7	4	9	3	—	•	57
35–44	•	•	•	•	8	15	10	8	8	—	•	49
45–54	•	•	•	•	•	8	12	11	8	—	—	39
55–64	•	•	•	•	•	•	8	16	3	—	—	27
65+	•	•	•	•	•	•	•	6	5	1	—	12
Total.....	—	5	34	34	33	34	38	57	28	1	—	264
Number Expected												
0–4	0.0	0.1	0.1	0.0	0.1	0.0	•	•	•	•	•	0.3
5–14	•	0.2	0.9	1.0	0.7	0.8	0.8	0.2	•	•	•	4.6
15–24	•	•	1.8	4.4	4.6	5.2	7.2	8.0	1.5	•	•	32.7
25–34	•	•	•	2.0	4.3	5.5	8.1	11.5	9.6	1.0	•	42.0
35–44	•	•	•	•	1.7	4.6	7.7	11.5	10.1	3.5	•	39.1
45–54	•	•	•	•	•	3.1	7.4	11.4	10.0	4.1	0.6	36.6
55–64	•	•	•	•	•	•	4.0	8.5	7.6	2.9	0.2	23.2
65+	•	•	•	•	•	•	•	3.7	5.0	1.9	0.3	10.9
Total.....	0.0	0.3	2.8	7.4	11.4	19.2	35.2	54.8	43.8	13.4	1.1	189.4

— Magnitude nil 0.0 Magnitude less than half unit employed • Category not applicable

TABLE 5a

MALE PATIENTS: OBSERVED AND EXPECTED NUMBER OF DEATHS AS CUMULATIVE FUNCTION IN PERCENTAGE IN LUPUS PATIENTS ADMITTED, BY DECADE OF ADMISSION, AGE AT ADMISSION, AND AGE AT DEATH

Decade of Admission	Age at Admission (years)	Age at Death (years)								
		<15	<25	<35	<45	<55	<65	<75	<85	<95
1895–1904	Number Observed									
	5–14	–	15.5	17.3	19.0	24.2	25.9	27.6	•	•
	25–34	•	•	5.3	21.6	40.9	59.9	81.6	84.2	84.2
	55–64	•	•	•	•	•	–	75.0	75.0	100.0
	Number Expected									
	5–14	1.2	5.1	10.3	14.1	20.5	29.9	32.1	•	•
	25–34	•	•	2.6	8.8	17.5	32.2	57.6	82.7	85.3
	55–64	•	•	•	•	•	13.5	48.7	84.3	100.0
1915–1924	Number Observed									
	5–14	2.0	6.0	6.0	9.9	13.8	•	•	•	•
	25–34	•	•	7.8	15.6	21.1	31.7	31.7	•	•
	55–64	•	•	•	•	•	43.3	53.3	80.0	80.0
	Number Expected									
	5–14	0.8	2.9	5.0	7.1	7.6	•	•	•	•
	25–34	•	•	1.6	5.8	12.7	23.1	25.5	•	•
	55–64	•	•	•	•	•	11.6	45.9	83.5	100.0
1935–1944	Number Observed									
	5–14	6.3	9.6	9.6	•	•	•	•	•	•
	25–34	•	•	7.1	19.8	19.8	•	•	•	•
	55–64	•	•	•	•	•	14.3	42.9	50.0	•
	Number Expected									
	5–14	0.4	1.5	1.6	•	•	•	•	•	•
	25–34	•	•	1.1	3.3	3.8	•	•	•	•
	55–64	•	•	•	•	•	10.0	35.3	40.5	•

– Magnitude nil • Category not applicable

TABLE 5b

FEMALE PATIENTS: OBSERVED AND EXPECTED NUMBER OF DEATHS AS CUMULATIVE FUNCTION IN PERCENTAGE IN LUPUS PATIENTS ADMITTED, BY DECADE OF ADMISSION, AGE AT ADMISSION, AND AGE AT DEATH

Decade of Admission	Age at Admission (years)	Age at Death (years)								
		<15	<25	<35	<45	<55	<65	<75	<85	<95
1895–1904	Number Observed									
	5–14	5.8	12.1	13.3	16.7	21.2	25.0	26.2	•	•
	25–34	•	•	8.3	23.2	30.5	38.3	66.1	87.4	88.3
	55–64	•	•	•	•	•	27.3	74.6	90.5	100.0
	Number Expected									
	5–14	1.4	5.2	10.3	14.2	19.9	27.7	29.4	•	•
	25–34	•	•	2.7	8.6	16.6	30.5	54.9	80.5	83.4
	55–64	•	•	•	•	•	10.9	43.9	82.1	100.0
1915–1924	Number Observed									
	5–14	3.3	12.5	15.8	17.5	18.4	•	•	•	•
	25–34	•	•	6.5	17.8	21.9	35.3	38.2	•	•
	55–64	•	•	•	•	•	16.2	63.4	86.7	98.5
	Number Expected									
	5–14	0.7	2.8	4.9	6.8	7.2	•	•	•	•
	25–34	•	•	1.9	6.3	12.5	21.0	23.0	•	•
	55–64	•	•	•	•	•	10.6	43.7	81.8	100.0
1935–1944	Number Observed									
	5–14	–	–	–	•	•	•	•	•	•
	25–34	•	•	3.1	3.1	5.6	•	•	•	•
	55–64	•	•	•	•	•	6.2	37.2	47.9	•
	Number Expected									
	5–14	0.3	1.0	1.1	•	•	•	•	•	•
	25–34	•	•	1.1	3.1	3.5	•	•	•	•
	55–64	•	•	•	•	•	8.9	32.2	37.2	•

– Magnitude nil • Category not applicable

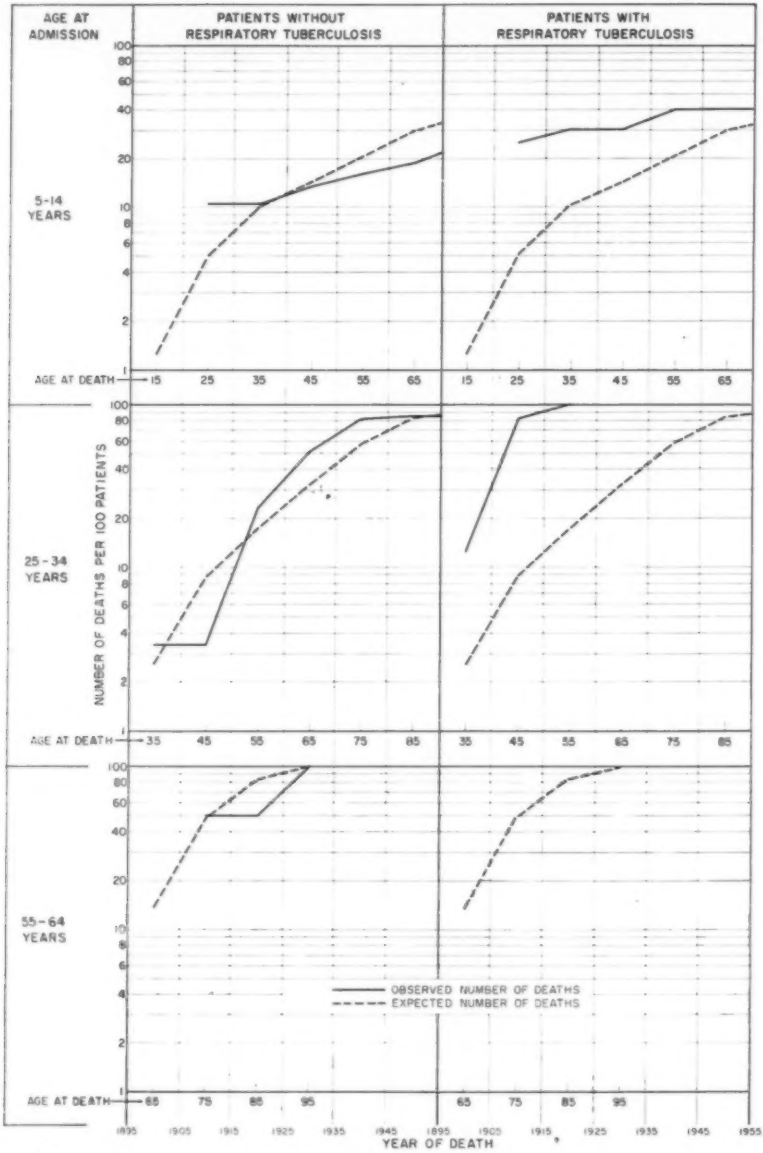

FIG. 5a. Male patients: observed and expected number of deaths, as cumulative function in percentage, in lupus patients admitted 1895–1904, by age at admission, age at death, and occurrence of respiratory tuberculosis.

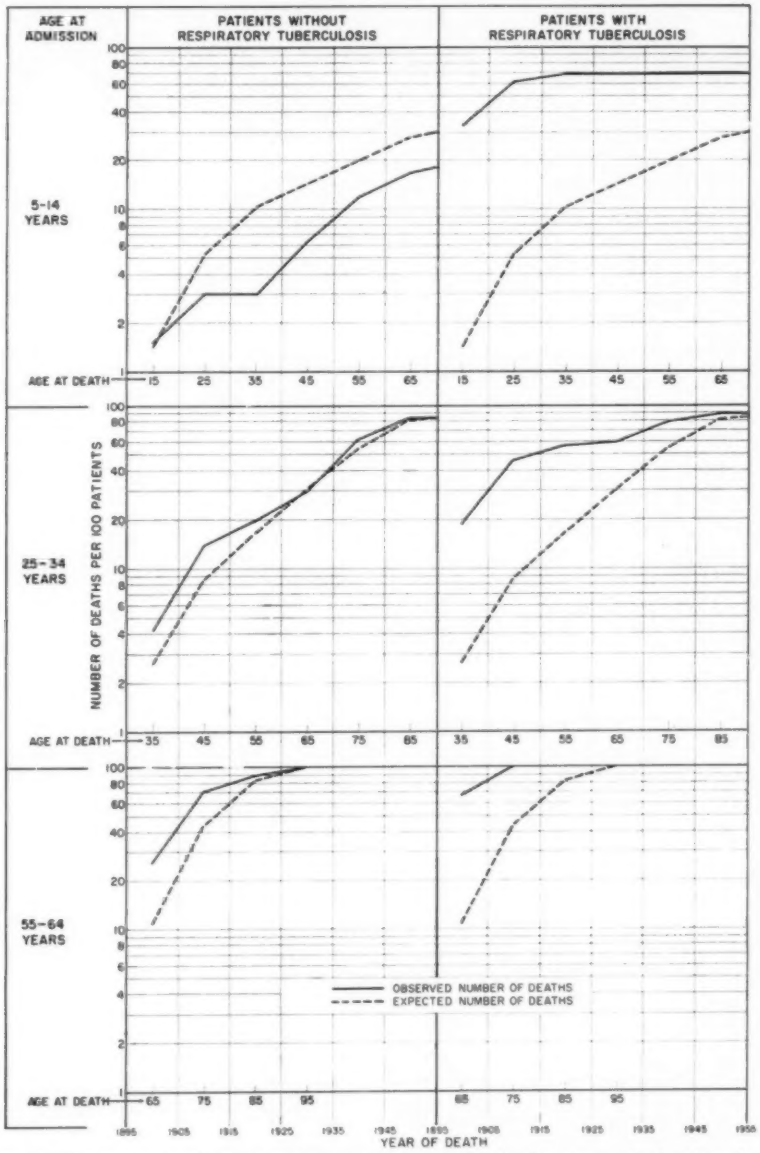

FIG. 5b. Female patients: observed and expected number of deaths, as cumulative function in percentage, in lupus patients admitted 1895–1904, by age at admission, age at death, and occurrence of respiratory tuberculosis.

TABLE 6*a*

MALE PATIENTS: OBSERVED NUMBER OF DEATHS AS CUMULATIVE FUNCTION IN PERCENTAGE IN LUPUS PATIENTS ADMITTED, BY DECADE OF ADMISSION, AGE AT ADMISSION, AGE AT DEATH, AND OCCURRENCE OF RESPIRATORY TUBERCULOSIS

Decade of Admission	Age at Admission (years)	Age at Death (years)								
		<15	<25	<35	<45	<55	<65	<75	<85	<95
1895–1904	*Lupus Patients*									
	Without respiratory tuberculosis									
	5–14	–	10.6	10.6	13.3	16.0	18.7	21.4	•	•
	25–34	•	•	3.4	3.4	23.4	50.1	80.1	83.5	83.5
	55–64	•	•	•	•	•	–	50.0	50.0	100.0
	With respiratory tuberculosis									
	5–14	–	25.0	30.0	30.0	40.0	40.0	40.0	•	•
	25–34	•	•	12.5	83.4	100.0	100.0	100.0	100.0	100.0
	55–64	•	•	•	•	•	–	100.0	100.0	100.0
1915–1924	*Lupus Patients*									
	Without respiratory tuberculosis									
	5–14	2.2	4.4	4.4	6.6	8.8	•	•	•	•
	25–34	•	•	•	3.4	10.1	23.9	23.9	•	•
	55–64	•	•	•	•	•	33.4	45.9	75.1	75.1
	With respiratory tuberculosis									
	5–14	–	25.0	25.0	50.0	66.7	•	•	•	•
	25–34	•	•	33.3	50.0	50.0	50.0	50.0	•	•
	55–64	•	•	•	•	•	50.0	50.0	50.0	50.0

– Magnitude nil • Category not applicable

TABLE 6*b*

FEMALE PATIENTS: OBSERVED NUMBER OF DEATHS AS CUMULATIVE FUNCTION IN PERCENTAGE IN LUPUS PATIENTS ADMITTED, BY DECADE OF ADMISSION, AGE AT ADMISSION, AGE AT DEATH, AND OCCURRENCE OF RESPIRATORY TUBERCULOSIS

Decade of Admission	Age at Admission (years)	Age at Death (years)								
		<15	<25	<35	<45	<55	<65	<75	<85	<95
1895–1904	*Lupus Patients*									
	Without respiratory tuberculosis									
	5–14	1.5	3.0	3.0	6.4	11.8	16.4	17.9	•	•
	25–34	•	•	4.2	14.0	19.7	29.5	61.0	88.1	89.4
	55–64	•	•	•	•	•	25.9	70.8	89.3	100.1
	With respiratory tuberculosis									
	5–14	32.2	60.8	68.0	68.0	68.0	68.0	68.0	•	•
	25–34	•	•	18.6	45.9	56.9	59.7	78.8	87.0	87.0
	55–64	•	•	•	•	•	66.7	100.1	100.1	100.1
1915–1924	*Lupus Patients*									
	Without respiratory tuberculosis									
	5–14	2.8	8.4	11.3	11.3	12.3	•	•	•	•
	25–34	•	•	2.5	9.7	14.5	29.0	31.3	•	•
	55–64	•	•	•	•	•	17.2	57.1	84.6	100.2
	With respiratory tuberculosis									
	5–14	6.3	41.7	48.0	64.7	64.7	•	•	•	•
	25–34	•	•	25.0	59.0	59.0	65.3	72.5	•	•
	55–64	•	•	•	•	•	12.5	100.0	100.0	100.0

• Category not applicable

have died, i.e., for the terminal fatality ratio to be computed. For the patients admitted 1895–1904, the prognosis computed a few years after admission is poorer for the age group 25 to 34 years than for the age group 55 to 64 years: the fatality ratio 10 years after admission was found to be 250 to 300 per cent for those admitted as children and young adults and about 150 per cent for the older patients. Assessed by the terminal fatality ratio, however, the prognosis is the same for the age groups 25 to 34 years and 55 to 64 years admitted 1895–1904. It is therefore clear that ratios computed at selected points in time can be misleading in the evaluation of the severity of a disease, and that the total curves (cumulative distribution functions) are the more accurate evaluation.

The fatality ratios for patients admitted at identical ages but at different periods of time are also illustrated in figure 4. Comparisons can be made only for the first 15 years after admission, this being the observation period for the last group admitted. For children and young adults the interval between the observed and expected number of deaths has increased relatively, i.e., the fatality ratio has risen, an observation that is taken to show that these patients have not experienced the same increase in survival as the general population.

The course of the disease in patients with histories of other forms of tuberculosis are illustrated in figure 5*a* and *b* and tables 6*a* and *b*. The number of patients is small; hence no distinction has been made between "lupus only" and "lupus plus nonrespiratory tuberculosis," a procedure warranted by the findings reported previously. It is clear from the figure that the over-all mortality rate of patients with lupus and possibly other nonrespiratory tuberculosis was the same as that for the general population. Among patients with respiratory tuberculosis, on the other hand, a steep rise is seen in the first years after admission, after which no increase or only slight increase is found. The highest fatality ratios are observed among children and young adults.

Discussion

The present study was designed to appraise the severity of lupus by the fatality ratio, a statistic which gives a comparison between the observed and expected number of deaths among the patients. The results have shown that the prognosis for patients in whom lupus was the only form of tuberculosis was good *quoad vitam*; these patients survived as the general population. Lupus has been very disfiguring in some cases, but nevertheless the disease in itself must be regarded as benign. However, most of the patients (nearly 80 per cent) suffered from other forms of tuberculosis, and the prognosis in these cases deteriorated, particularly when respiratory tuberculosis occurred.

The fatality ratios given are computed on the basis of the majority of all lupus patients admitted within the period 1895–1954, and can accordingly be taken to apply to the whole series of patients admitted. This holds true even if the ratios for those excluded from the study differ essentially from the ratios observed; such a difference, however, is not likely.

There have been few patients with lupus in the general population, and the results can thus be taken to show, as well, the prognosis for lupus patients as compared with that for persons without the disease.

Fatality ratios by the temporal relation of lupus to other forms of tuberculosis have also been analyzed. The groups were demarcated according to whether lupus occurred before, simultaneously with, or after other forms of tuberculosis, although the material is not correctly delimited for the use of criteria relevant to the clinical onset of the disease. However, if there had been large differences in fatality ratios for the various groups, the investigation would have disclosed them, and the analysis is taken as valid. *A priori* a lower fatality ratio was expected among the patients in whom lupus was the initial tuberculous disease, as their status might be likened to BCG-vaccinated persons: the virulence of tubercle bacilli from lupus lesions is generally low, sometimes equal to the virulence of BCG strains (4–7). However, there was no significant difference (at the 5 per cent level) between fatality ratios for the different groups; in fact a tendency for *higher* ratios in patients in whom lupus was the initial tuberculous disease was observed. In parallel, it may be mentioned that the incidence of respiratory tuberculosis was five times greater in patients with an initial lupus than in the general population (1).

It might be assumed, as well, that fatality ratios for patients with respiratory tuberculosis

and lupus were lower than ratios for patients with respiratory tuberculosis only, lupus providing an immunization (8). This problem cannot be investigated at the present time as fatality ratios are not available for patients with respiratory tuberculosis only.

The technique of the fatality ratio is considered of particular interest, not only to the public health worker, but also to the clinician for evaluation of treatment regimens (9, 10). In the present material, fatality ratios are computed for a series of patients, practically none of whom has received chemotherapy. Within a few years fatality ratios will be available for patients admitted to such treatment. It will then be possible to use the fatality ratio to determine the efficacy of the treatment: the lower the ratios, the higher the efficacy.

Fatality ratios are computed independently of statements of cause of death, an important advantage over other methods often used in public health and clinical work to evaluate the seriousness of diseases. The physicians may be biased in stating cause of death, and this error can easily be so large that it obscures the efficacy of treatment or, conversely, creates a false impression of effectiveness. The independence of statements of cause of death also facilitates comparisons between different countries and various periods of time.

Summary

The fatality ratio is defined as the observed number of deaths among patients in proportion to the number expected, i.e., the number which would occur if the patients had experienced the same mortality as the general population. The method is regarded of importance not only to the public health worker, but also to the clinician comparing the efficacies of different treatment regimens.

Fatality ratios have been calculated for 3,313 patients with lupus vulgaris cutis admitted to the Finsen Institute, Copenhagen, from 1895 through 1954.

Of the 980 male patients, 437 died. The expected number of deaths was 346, and the summary fatality ratio was 126 per cent. Of the 2,333 female patients, 1,171 died. The expected number of deaths was 1,046 and the summary fatality ratio was 112 per cent. Far more deaths occurred than were expected from childhood until thirty-five years of age: the ratios varied about 250 to 300 per cent. This reveals that in the older age groups the observed number of deaths was lower than the expected number.

When lupus was the only form of tuberculosis, the life expectancy of patients was not influenced. However, in nearly 80 per cent of the patients, other forms of tuberculosis were present, and in these cases the prognosis deteriorated (the fatality ratios were raised). This was especially marked when respiratory tuberculosis was present. Whether lupus occurred before, simultaneously with, or after other forms of tuberculosis was not observed to alter the prognosis.

A much larger number of patients than expected died within the first years after admission, especially children and young adults.

Sumario

El Coeficiente de Letalidad para el Lupus Vulgar del Cutis

El coeficiente de letalidad queda definido como el número de muertes observado en los enfermos en proporción al número esperado, es decir, el número que hubiere habido si los enfermos hubiesen experimentado la misma mortalidad que la población general. El método se considera importante no sólo para el higienista sino también para el clínico que compara la eficacia de distintos regímenes terapéuticos.

Se han calculado los coeficientes de letalidad para 3,313 enfermos de lupus vulgar del cutis recibidos en el Instituto Finsen, de Copenhague, de 1895 a 1954.

De los 980 varones, fallecieron 437. El número esperado de muertes era 346 y el coeficiente de letalidad sumarizado fué de 126 por ciento. De las 2,333 mujeres, fallecieron 1,171. El número esperado de muertes era 1,046 y el coeficiente de letalidad sumarizado fué de 112 por ciento. Hubo muchas muertes más que las esperadas desde la niñez hasta la edad de treinta y cinco años: los coeficientes variaron aproximadamente de 250 a 300 por ciento. Esto revela que, en los grupos de edad mayor, el número observado de muertes fue menor que el esperado.

Cuando el lupus era la única forma de tuberculosis, no se afectaba la expectativa de vida de los enfermos. Sin embargo, en casi 80 por ciento de éstos existían otras formas de tuberculosis, y en estos casos empeoraba el pronóstico (elevando el coeficiente de letalidad). Esto se destacó en particular cuando había tuberculosis del aparato respiratorio. No se observó que se alterara el pronóstico ya existiera el lupus antes, simultánea-

mente, o después de las otras formas de tuberculosis.

Un número mucho mayor de enfermos que el esperado, en particular niños y jóvenes, falleció en el primer año consecutivo al ingreso.

Resume

Rapport de mortalité pour le lupus tuberculeux

La rapport de mortalité est défini comme le nombre de décès enregistrés parmi les patients proportionnellement au nombre prévu, c'est-à-dire le nombre obtenu si la mortalité avait été la même dans ce groupe que dans la population générale. La méthode est considérée importante non seulement pour les techniciens d'Hygiène Publique, mais aussi pour le clinicien lorsqu'il compare l'efficacité de différents programmes de traitement.

Les rapports de mortalité ont été calculés pour 3313 patients atteints de lupus tuberculeux hospitalisés à l'Institut Finsen de Copenhague entre 1895 et 1954.

Parmi 980 patients du sexe masculin, 437 sont morts. Le nombre de décès prévu était de 346 et le rapport de mortalité d'ensemble était 126%. Parmi 2,333 patients du sexe féminin, 1,171 sont mortes. Le nombre de décès prévu etait 1,046 et le rapport de mortalité d'ensemble etait 112%. Depuis l'enfance jusqu'à l'âge de 35 ans le nombre de décès était beaucoup plus élevé qu'on ne le prévoyait, les rapports variaient d'environ 250 à 300%. Ce qui a révélé que dans les groupes plus âgés le nombre des décès observés était inférieur au nombre prévu.

Lorsque le lupus était l'unique forme de la tuberculose, la durée probable de la vie des malades n'était pas influencée. Toutefois, chez presque 80% des malades, il existait d'autres formes de tuberculose et, dans ces cas, le pronostic était aggravé (les rapports de mortalité étaient augmentés). Ceci était spécialement marqué quand la tuberculose affectait les voies respiratoires. Que le lupus se produise avant, en même temps ou après d'autres formes de la tuberculose le pronostic n'en est pas modifié. Le nombre de malades ayant succombé dans les premières années qui ont suivi l'admission à l'hôpital, a été beaucoup plus élevé que le nombre prévu, spécialement parmi les enfants et les jeunes adultes.

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VACCINATION AGAINST TUBERCULOSIS WITH NONLIVING VACCINES

I. The Problem and Its Historical Background

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REVIEW OF LITERATURE—*concluded*

1951–1955

Sadelkow (1951) postulated that the relative resistance of children to tuberculosis stemmed from their continuous ingestion of tubercle bacilli "weakened" ("abgeschwächt") by sunlight (201). He vaccinated 2 (!) guinea pigs with ultraviolet-irradiated bacilli and six weeks later fed them dandelion leaves dipped in a culture of living virulent tubercle bacilli. The animals apparently remained well.

Purified tuberculoprotein and -polysaccharide preparations were reported by Seibert (1950) to be devoid of immunogenic activity (202).

In a study of the effects of cortisone on tuberculosis in mice, Solotorovsky, Gregory, and Stoerk (1951) also investigated the effect of the steroid on animals vaccinated prior to infection with steam-killed human tubercle bacilli (203). Each animal received six intraperitoneal injections of 0.5 ml. each of the steamed culture and was challenged intravenously two days after the last injection. The vaccinated animals survived longer than the unvaccinated controls if they were not subjected to cortisone; cortisone overcame the vaccination-induced protection.

A comprehensive investigation of antituberculosis vaccines killed by a variety of methods—heat, ultraviolet irradiation, urea, and extreme pH—was reported in 1951 by Swedberg (204). Mice and guinea pigs were employed, and in most of the experiments survival time after infection was used to measure the degree of resistance. Large numbers of animals were used, and the results were subjected to statistical analysis. It was again found that killed vaccines prepared from different strains of tubercle bacilli differed in their immunogenic activity, heat-killed BCG proving inferior to heat-killed human tubercle bacilli. The pH of the suspension in which tubercle bacilli were killed by heat was shown to influence the immunizing capacity of the product. Swedberg concluded that "heat-killed human tubercle bacilli in big doses and dispersed in paraffin oil reveal the same unquestionable immunizing effect as living BCG." The subcutaneous route of vaccination with killed vaccines was found to be a satisfactory one, and the high protective activity of killed vaccines was seen even when the animals were challenged by the intravenous route.

Yanagisawa, Kanai, Shiga, and Ito (1951) protected guinea pigs by vaccination with 0.1 mg. of heat-killed BCG suspended in paraffin, or with 0.1 ml. of a paraffin extract of killed BCG, to the same extent against subsequent tuberculous infection as was possible with living BCG (205). The degree of protection in their experiments was evaluated by comparing the extent of systemic tuberculous disease of the vaccinated and control animals sacrificed at intervals after infection. Yanagisawa and Kanai (1952) also claimed a protective effect for a tuberculin preparation administered intramuscularly in oil (206), but the reported tuberculin-sensitizing properties of this preparation suggest that it may have been contaminated with intact bacillary bodies. Kanai and his co-workers (1952) vaccinated school children in Japan with heat-killed tubercle bacilli suspended in peanut or paraffin oil by the scarification method (207); local ulcerations did not develop.

Birkhaug and Darricarrere (1952) reinvestigated the ultraviolet-killed vaccines prepared by Sarber's (*see* 200) and Milzer's (*see* 199) groups (208). The preparations employed by Birkhaug and Darricarrere were three to fifteen months old at the time of testing. Groups of 18 to 24 guinea pigs were given three intraperitoneal injections of 5 mg. each, and were challenged one to three months later by the intramuscular route. Three-and-a-half months after infection, the survivors were killed, and the extent of tubercu-

lous disease was ascertained by visual inspection. Although the extent of disease was less in the animals which had received the killed vaccines than in the nonvaccinated control groups, the retardation of the disease process was greater in the guinea pigs vaccinated with living BCG. The investigators observed extreme "irritability and distress" in the animals after vaccination with the killed vaccine, and ascribed this to the presence of merthiolate in the preparations. That this intoxication was severe (and may therefore have obscured a potentially much greater immunogenic effect of the irradiated vaccines!) is indicated by the observation that 27.1 per cent of the animals given the killed vaccines succumbed within four weeks after vaccination, whereas only 8.3 per cent of those that received living BCG died during the same period. In a later experiment, Birkhaug and Darricarrere (1953) found that heat-killed tubercle bacilli possessed little immunogenic activity compared with living BCG (209).

Helmert (1952) vaccinated groups of 30 guinea pigs each with living BCG, heat-killed human tubercle bacilli, and heat-killed human tubercle bacilli which had been extracted with petroleum-ether (210). Vaccination was done by the intramuscular route, as was challenge infection six weeks later. The various vaccines were equally effective in prolonging the survival time of the animals over that of a nonvaccinated control group, and Helmert seriously questioned the common acceptance of living BCG as a satisfactory vaccine in view of its no-greater efficacy (but definite disadvantages) compared with nonliving vaccines.

Nonliving antituberculosis vaccines have received serious laboratory and clinical consideration and trial by several groups of investigators in Italy. The two preparations studied most extensively have been a formol-killed mixture of human, bovine, and avian strains of tubercle bacilli, known as Anatuberculina Integrale Petragnani (AIP) and a heat-killed preparation of tubercle bacilli to which hyalouronidase is added, known as Vaccino Diffondente Salvioli (VDS).

The older literature on AIP vaccination has recently been reviewed by De Toni (1953) (211), and by Buonomini and Santopadre (1954) (212). Recently, Buonomini and Santopadre reported this vaccine to be equally as effective as living BCG in increasing the survival time of guinea pigs infected subcutaneously with virulent tubercle bacilli several months after vaccination by the intradermal multipuncture method (212). These workers stressed the necessity of employing statistically significant numbers of animals and rigorously controlled experimental conditions in order to obtain repeatable and significant results in vaccination studies.

Further work in several hundred guinea pigs led Buonomini and his collaborators to conclude that AIP's efficacy was consistently as good as, or slightly better than, that of living BCG, even when the killed vaccine was given intradermally (213). (The demonstration that the intradermal route is satisfactory for vaccinating with nonliving vaccines is extremely important because the proponents of living BCG have often argued that, although nonliving vaccines can be shown to be effective when administered by routes not feasible for use in man, they are ineffective when given by safe and convenient intradermal methods.)

The VDS vaccine was developed by Salvioli (1953), who found that hyaluronidase facilitated the translocation of killed tubercle bacilli from the site of vaccination, thus increasing the likelihood that sufficient quantities of the vaccine would reach sites of the body involved in the development of protective immunity (214). Salvioli, Esposti, and Dina (1952, 1953) found that intradermal vaccination with small quantities of AIP and VDS gave greater protection to dogs than living BCG (215, 216). In an experiment involving 39 puppies, only 21.4 per cent of the animals vaccinated with killed vaccines succumbed after challenge infection as compared with a tuberculosis mortality rate of 37.5 and 100 per cent, respectively, in BCG-vaccinated animals and in nonvaccinated controls (216). Esposti (1954) further reported that hyaluronidase enhanced the protective efficacy of heat-killed tubercle bacilli in rabbits (217).

More than 100,000 children have been vaccinated in Italy with intradermally injected quantities of AIP or VDS (218, 219). The results of this program in the Bologna area, where careful follow-up study of more than 18,000 vaccinated infants was possible, have been summarized by Salvioli as follows:

> Our vaccinations have given results we had never hoped for . . . in the department of

> Bologna alone these results have been collected in more than 18,000 newborn, vaccinated and followed up for a period of from two to 18 years . . . Subjects vaccinated at birth with four injections of Anatubercolina presented after two to 16 years tubercular manifestations which were notably reduced in quality and quantity as compared to non-vaccinated children of the same age. In 100 families in which there was a history of . . . tubercular activity, the children vaccinated from one to 13 years previously presented, in comparison with non-vaccinated siblings, unimportant lesions; there were no tisiogenic or disseminated cases, and no lethality (among them), while in the unvaccinated siblings there was a five per cent mortality and a 12 per cent incidence of tisiogenic and disseminated lesions. (219)

The protective effect of VDS in heavily exposed infants was also reported by Grandi and Malossi (1956) (220).

Although most Italian investigators in the field of tuberculosis prevention have found AIP and VDS at least as effective as living BCG, some Italian workers have reported these killed vaccines to be inferior (221, 222). On the whole, however, the Italian experiments with formol- and heat-killed tubercle bacilli provide persuasive evidence of the efficacy of nonliving vaccines in experimental animals and in man.

Sigurdsson (1952), working in Iceland, vaccinated a total of 289 lambs over a three-year period with 5 to 50 mg. of heat-killed *M. johnei* by the subcutaneous route (223). A group of 266 nonvaccinated animals on the same farms served as controls. Over an observation period of one and a half to three and a half years, 16 of the control, but none of the vaccinated, animals died of Johne's disease. At the end of the observation period all animals still alive were sacrificed and carefully examined for the presence of paratuberculotic lesions. These were found in the intestines of 21 control, but of only 4 vaccinated, animals. This study again shows the efficacy of a nonliving vaccine against a tuberculosis-like disease under "natural" field conditions.

Yaoi, Takei, and Saito (1952) reviewed the Japanese literature on vaccination with killed antituberculosis vaccines, and presented the results of their own studies on vaccination of guinea pigs with human tubercle bacilli killed by exposure to 0.1 per cent merthiolate (224). Groups of 5 to 10 guinea pigs each were employed in the study, each animal receiving one or more intraperitoneal injections of the killed vaccine or of living BCG. Challenge infection took place by the subcutaneous route six weeks to five months later. Living BCG was found to be only "a little superior" in retarding the tuberculous process, as seen by post-mortem examination of animals sacrificed six weeks after infection. The killed vaccine was found to retain its activity after several months of storage, and the writers highly recommended the use of nonliving vaccines for the protection of cattle and, eventually, for use in man. Abe (1953) vaccinated young mice subcutaneously and intraperitoneally with 1 mg. quantities of heat-killed tubercle bacilli suspended in saline or oil, or with living BCG; a paraffin extract of tubercle bacilli was also tested (225). The killed vaccines prolonged the life of the animals infected intravenously five to six weeks after vaccination, and retarded the multiplication of the infecting bacilli in their organs. Living BCG proved to be superior, however.

Baldwin, Gilbert, Iland, and Jones (1953) vaccinated mice with a dialyzed urea extract of tubercle bacilli, reporting a prolongation of their lives following heavy intravenous challenge two weeks after vaccination (226). Guinea pigs given two subcutaneous injections of 5 mg. each of intact, urea-killed tubercle bacilli at eight-day intervals were reported by Iland to show the same degree of heightened resistance to subcutaneous infection one month later as animals vaccinated with freshly prepared living BCG (227). Groups of 15 to 20 guinea pigs each were employed in this experiment. When the urea-killed vaccine was tested in red mice, however, Iland and Jespersen found its protective activity to be lower than that of living BCG (228). They reported the urea-killed vaccine to be more effective than a heat-killed preparation, and found that the addition of paraffin enhanced the efficacy of the urea-killed vaccine. Also employing red mice, Jespersen and Magnusson (1956) found heat-killed human tubercle bacilli and heat-killed BCG equally effective in prolonging the life of animals infected intravenously with virulent bovine tubercle bacilli forty-two days after vaccination (229). The vaccinations were administered subcutaneously in 1 mg. (dry weight) quantities. Mice vaccinated with living BCG exhibited a greater degree of resistance. The experiments were statistically significant.

An investigation of nonliving antituberculosis

vaccines in mice, designed to establish the optimal methods and conditions of vaccination, was recently initiated by Dubos, Schaefer, and Pierce (1953) (230), and has been continued by Weiss and Dubos (231–234) and by Weiss and Wells (235). The results obtained may be summarized as follows:

> Intact phenol-killed, acetone-washed tubercle bacilli of virulent, avirulent, or attenuated strains can bestow a degree of protective immunity on mice equivalent to that given by living BCG. This immunity is manifested by a prolonged survival time after infection, an inhibition of the multiplication of the infecting bacilli in the organs of the animals, and their better physical condition in the early periods after infection. The degree of immunity which can be elicited depends on the dose, route, and vehicle of the vaccine. The optimal dose falls within a narrow range, from 1 to 3 mg. (dry weight), an antigenic quantity, which, though small, exceeds that contained in the usual vaccinating doses of *living* BCG many times (235).
>
> Several adjuvant substances, notably sphingomyelin, typhoid somatic lipopolysaccharide, and Freund's incomplete oil adjuvant, enhance the immunogenicity of the phenolized vaccine. The toxigenic and immunogenic activities of killed tubercle bacilli appear to be associated with distinct and separable cellular moieties; either of these properties can be reduced or abolished, without affecting the other, by various chemical treatments of the intact killed bacilli. A methanol-soluble extract of dried, phenolized, acetone-washed tubercle bacilli appears capable of eliciting a similar degree of protection, but somewhat larger quantities, 3 to 4 mg., are necessary. (The procedure of extraction liberates only about 12 per cent of the cellular constituents of tubercle bacilli, so that each milligram of methanol extract represents about 8 mg. of intact killed bacilli, and the optimal doses of the extract, 24 to 32 mg. of intact bacilli. The extract thus possesses only a fraction of the immunogenically active components of tubercle bacilli, or, alternately, must be considered to have lost much of its immunogenic activity in the preparatory process.) Combination of the methanol extract with small quantities of the extraction-residue enhances the efficacy of either component synergistically. The duration of the immunity bestowed by the extract is at least as long as that given by living BCG.

The results obtained in the mouse experiments were deemed sufficiently encouraging to justify a full investigation of the activity of phenol-killed tubercle bacilli and their methanol extracts in other species of animals. A series of experiments, involving more than 1,800 guinea pigs and a number of cattle, were accordingly initiated by Weiss and his co-workers in England during the years 1955 to 1957. Several aspects of the results of the guinea pig studies have already been communicated briefly (236) and a more detailed report will be made shortly (237, 238). The conclusions drawn from the guinea pig experiments corroborate those made from the mouse studies and provide statistically valid proof that killed vaccines can provide a degree of protection, *when administered under empirically determined satisfactory conditions*, of the same order as that given by living BCG. Effective immunizing doses did not induce toxic manifestations in guinea pigs, and it appeared possible to elicit immunity with methanol extract preparations without rendering the animals tuberculin skin sensitive.

Seagle, Karlson, and Feldman (1953) found ultraviolet-killed tubercle bacilli ineffective as a vaccine in guinea pigs (239). The animals received 1.5 mg. of the preparation (apparently moist weight) by a variety of routes. Only a few of the vaccinated animals became tuberculin positive, however, thus raising some doubt whether immunologically effective quantities of the vaccine were administered in such a manner that they reached immunologically active sites in the animals.

Smith, Grover, and Nungester (1953) tested ultraviolet-killed tubercle bacilli and a number of bacillary fractions in mice, rats, and guinea pigs (240). The vaccines were administered subcutaneously, and the animals were infected one month later by the intraperitoneal or intravenous routes. Two months after infection, the animals were sacrificed, and the extent of tuberculous disease was compared with that of control animals. Guinea pigs and rats vaccinated with small quantities, 0.2 to 2.0 mg., of some of the killed preparations showed the same degree of inhibition of the tuberculous disease process as animals given equal amounts of living BCG. In contrast, one of the lipid fractions not only failed to bestow a degree of immunity, but actually lowered resistance, thus again indicating that the immunogenicity of certain nonliving vaccines may be masked by immunogenically inhibitory components. Neither living nor nonliving vaccines bestowed a significant degree of protection on mice under the conditions of this experiment.

Further studies by Smith and Kubica (1955) again provided evidence of the fact that tubercle bacilli possess greater immunogenic activity when some of their lipid constituents are removed (241). Very small quantities of tubercle bacilli exhaustively extracted with neutral organic solvents (as little as 0.05 mg., given subcutaneously in Freund's incomplete adjuvant) bestowed a greater degree of protection on guinea pigs against experimental tuberculous infection three to four weeks later than intact killed tubercle bacilli, and proved to be as effective as living BCG. The immunogenicity of the defatted bacilli was partially destroyed by autoclaving. That certain lipid fractions of tubercle bacilli are, on the other hand, necessary for the induction of immunity was evidenced by the fact that further extraction of the "defatted" bacilli with acidic alcohol-ether mixtures destroyed their immunizing capacity; a crude chloroform extract of the bacilli was also found to have immunogenic properties.

A paraffin extract of heat-killed tubercle bacilli and intact heated bacilli was found by Yamada (1954) to be as effective as living BCG (242). Vaccination was performed by subcutaneous injection or by the scarification method, and infection was established by the subcutaneous route four to six weeks later. The animals were sacrificed ten weeks after infection, and the criterion of resistance was based on the relative extent of disease in vaccinated and control animals.

Aufdermaur and Brodhage (1955) reported living BCG to be superior to urea-killed tubercle bacilli in guinea pigs (243). Only a few animals were employed in each vaccination group, however, and the writers themselves point to this as a limitation of the significance of their study. They nevertheless expressed the opinion that living BCG is the best available antituberculosis vaccine, despite its potential hazards and instability.

Bloch and Segal (1955), Palmer, Ferebee, Meyer, and Bloch (1955), and Meyer (1956) compared the immunizing efficacy of living BCG and tubercle bacilli killed by heat, phenol, or ultraviolet irradiation in a large number of mice and guinea pigs (244–246). The living vaccine was consistently found to be superior. These experiments, admirably designed and supervised in most respects are, however, open to one major question, which, in the opinion of Weiss and Wells (235), may invalidate the comparison of living and nonliving preparations. In attempting to relate the dosage of both types of vaccine, the nonliving preparations were given in multiples of the living one, up to one hundred times the antigenic mass contained in a "standard dose" of living BCG being employed. This standard dose was 0.075 mg., *wet weight*, suspended in 0.1 ml. Thus, the largest amount of killed vaccine was 7.5 mg., *wet weight*, or in the vicinity of 1 mg., *dry weight*. Moreover, a portion of this inoculum is likely to have been lost in the discharge of material from the draining vaccinal lesions which were reported to have developed in many of the animals. While other workers have indeed succeeded in bestowing a degree of immunity against tuberculosis with very small quantities of certain killed preparations, the possibility that the doses of the vaccine employed by Bloch and Meyer's group fell short of those necessary to elicit a maximal degree of protection with intact killed tubercle bacilli is a very real one.

Takeda and Kiuchi (1955) found that a mixture of various extracts of killed tubercle bacilli had a protective effect on guinea pigs against experimental tuberculous infection thirty-four days after intramuscular vaccination (247). The protection was evident from the better physical condition of the animals after infection and the retardation of the tuberculous disease process as seen at post-mortem examination of sacrificed animals.

A degree of survival protection was bestowed on guinea pigs by Vahlne, Sievers, and Nygren (1955), employing a phenol-killed, hexane-extracted preparation of tubercle bacilli (248). The animals were given three subcutaneous injections of an emulsion of the vaccine at one-week intervals and were infected by the same route thirty-six days later. The experiment was discontinued after seventy-five days because the animals developed draining tuberculous abcesses at the site of infection. In another experiment, the vaccine was found to bestow a degree of survival protection on a few rabbits. Living BCG appeared to bestow a greater degree of resistance in these experiments, but their early termination robs the comparisons of real value because animals vaccinated with nonliving vaccines sometimes show a superior degree of resistance in the *later* stages of tuberculous disease than those given living BCG (237, 244).

Youmans, Millman, and Youmans (1955) ground living avirulent human tubercle bacilli in a ball mill and separated a number of fractions by high-speed centrifugation of the disrupted cellular mass (249). A particulate, enzymatically active fraction known as the "red fraction" was capable of eliciting the same degree of survival protection in mice as a large inoculum of living bacilli of the same avirulent strain, H37Ra. By determining the degree of immunity bestowed on mice by different doses of living H37Ra, the writers concluded that the activity of the red fraction could not be accounted for entirely by contamination with small numbers of intact living bacilli. The possibility that such contaminating bacilli exerted an adjuvant effect on the fraction cannot be excluded, however, and it must also be noted that in the hands of some investigators, notably Bloch and Segal (244), very small numbers of living H37Ra are found capable of eliciting a significant degree of immunity. It is not entirely certain, therefore, that Youmans and his group were dealing with a *bona fide* nonliving vaccine, especially as their preparation was readily inactivated by heat, lyophilization, and sonic vibration (250).

1956–1958

Working with a phenol-killed preparation of tubercle bacilli prepared by Weiss (232), Dworski (1956) undertook a careful study of its immunizing efficacy in guinea pigs, employing 25 animals per vaccination group (251). The animals received four subcutaneous injections of 6.25 mg. (dry weight) each of vaccine suspended in saline, or four injections of 0.625 mg. each in Freund's adjuvant. Another group of animals received living BCG intradermally. All animals were infected subcutaneously with 160,000 virulent tubercle bacilli fifty-eight days after the last vaccination. The animals which had received the killed preparations survived significantly longer than a group of nonvaccinated controls, and sample animals sacrificed at intervals after infection were found to have fewer and less widely dispersed tuberculous lesions, but the protection was of a lower order than that given by living BCG. The apparent discrepancy between these findings and Weiss' observations that intact phenolized tubercle bacilli are as effective in the guinea pig as living BCG (236, 237) may well be due to differences in Dworski's experimental conditions, especially with regard to dosage of vaccine, and numbers, strain, and route of infecting bacilli.

Nitrous acid was used by Yoshimura (1956) to kill tubercle bacilli of a virulent strain (252); the use of nitrous acid as a killing agent was based on reports of its employment in the inactivation of various biologic substances (253). Groups of 7 guinea pigs each, of approximately the same weight, received by the intraperitoneal route 5 mg. (dry weight) of this preparation, of living BCG, or of tubercle bacilli boiled for thirty minutes. The animals were infected three weeks later by a subcutaneous injection of virulent human tubercle bacilli. Two months after infection they were killed and the extent of tuberculous disease was estimated by the Feldman Index scheme. The bacilli killed by nitrous acid were found to have bestowed as high a degree of enhanced resistance as living BCG, or higher, but the boiled preparation was ineffective. Treatment of tubercle bacilli with nitrous acid for eight hours provided a more effective preparation than treatment for two or sixteen hours, thus again pointing to the necessity for an empirical approach to determine the most favorable method of preparing a killed vaccine. Mukozaka (1957) repeated these experiments in guinea pigs and reported similar findings: a vaccine prepared from a nitrous-acid–killed human strain of tubercle bacilli was found to be superior to living BCG (254).

Hoyt, Knowles, Moore, and Smith (1957) (255), Hoyt, Moore, Knowles, and Smith (1957) (256), and Hoyt, Dennerline, Moore, and Smith (1957) (257) studied the effects of vaccination with BCG killed by mild heating (70°C. for one hour) in mice. Subcutaneous or intraperitoneal vaccination of the animals with quantities of vaccine as small as 0.2 mg. prolonged their survival time after intravenous infection. The actitivy of the killed vaccine was enhanced by n-hexadecane, this substance having no immunogenic activity when given alone. A waxy fraction of tubercle bacilli also proved capable of prolonging survival time after infection, but this activity seemed to be dependent on the presence of small quantities of contaminating bacillary debris. Large numbers of animals were used in this study, and the results have statistical significance. It is interesting to note that Hoyt and his associates found vaccination to be con-

siderably more effective in male than in female animals.

The mouse corneal assay method, developed by Rees and Robson, and Robson and Sullivan (258, 259), was used by Acharya, Sullivan, and Robson (1958) to measure antituberculosis immunity in mice following intradermal or intracorneal vaccination with living and phenol-killed tubercle bacilli (260). Resistance is assessed in this method by the extent of bacillary multiplication and the development of microscopic lesions in the infected eye. The workers concluded that the phenolized preparation, when given in large doses, produced an immunity almost comparable with that seen following vaccination with living tubercle bacilli.

Hedgecock (1958) studied the effect of diet on the development of acquired immunity to tuberculosis in mice (261). His observations provided convincing evidence of the high degree of efficacy of small quantities of a nonliving vaccine, even against a massive challenge infection. Tubercle bacilli of BCG and human strains were killed with ethylene oxide. Approximately one million dead bacilli were given to the animals intraperitoneally—a very small antigenic quantity. Comparable groups of 15 mice each were vaccinated with living BCG. Infection took place five to 11 days later with a large number of virulent tubercle bacilli (0.05 ml. of a five-day-old culture) injected intravenously. The median survival time of the animals vaccinated with the

TABLE 1

THE VARIOUS METHODS OF PREPARATION OF THE NONLIVING VACCINES DISCUSSED IN THIS REVIEW*

Method of Preparation	Reference Number
Tubercle bacilli killed by heat	13, 15, 16, 17, 24, 27, 29, 32, 41, 45, 54, 58, 61, 62, 63, 64, 65, 66, 67, 68, 69, 91, 92, 93, 94, 95, 96, 97, 98, 100, 101, 103, 104, 110, 112, 117, 119, 120, 121, 122, 123, 124, 128, 134, 139, 140, 142, 143, 144, 145, 146, 148, 151, 152, 155, 156, 157, 158, 159, 160, 162, 163, 166, 167, 168, 169, 170, 171, 172, 173, 174, 176, 177, 178, 179, 181, 182, 183, 187, 193, 195, 196, 197, 203, 204, 205, 207, 209, 210, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 225, 228, 229, 242, 244, 245, 246, 255, 256, 257
Tubercle bacilli killed by exposure to sunlight or ultraviolet irradiation	16, 25, 47, 129, 180, 193, 198, 199, 200, 201, 204, 208, 239, 240, 244, 245, 246
Tubercle bacilli permitted to die spontaneously after prolonged storage or incubation in aerobic or anaerobic environments	16, 39, 59, 66, 67, 68, 69, 79, 80, 129, 147, 162, 185, 186
Tubercle bacilli found to be dead after exposure to serum or organ emulsions	34, 35, 49, 55, 56, 113, 124, 125, 134, 135
Tubercle bacilli killed with formol	152, 157, 160, 166, 178, 211, 212, 213, 215, 216, 218, 219, 221, 222
Tubercle bacilli killed with phenol	16, 164, 230, 231, 232, 233, 235, 236, 237, 244, 245, 246, 251, 260
Tubercle bacilli killed with urea	31, 204, 227, 228, 243
Tubercle bacilli killed by other chemical agents or physical means	17, 27, 32, 37, 38, 40, 45, 46, 60, 63, 127, 194, 224, 249, 250, 252, 254, 261
"Tuberculins," culture filtrates, and fractions and extracts of tubercle bacilli	14, 17, 21, 23, 26, 28, 29, 33, 36, 39, 45, 48, 51, 52, 53, 57, 63, 64, 65, 70, 82, 83, 84, 85, 86, 87, 88, 98, 99, 105, 106, 107, 108, 109, 112, 115, 130, 131, 137, 141, 150, 161, 165, 175, 181, 184, 187, 189, 190, 191, 202, 205, 206, 210, 225, 226, 233, 234, 235, 236, 238, 240, 241, 242, 247, 248, 263

* Only those references giving some description of the vaccine preparation are included in this table.

killed organisms exceeded that of those which had received living BCG. In a typical experiment, the median survival time of unvaccinated control animals was 27 days; of the living BCG group, 78 days; and of a killed-vaccine group, 85 days; when the animals were fed a special diet, the respective median survival figures were 140, 181, and 216 days.

* * *

The various nonliving vaccines which have been discussed are grouped in table 1 according to their method of preparation. It is clear at a glance that by far the greatest attention has been given to heat as a method of killing. This would not seem, however, to be the one most likely to guarantee the full retention of activity by an immunologic preparation, and the several reports of reduction or loss of immunogenicity by tubercle bacilli after prolonged heating point in this direction. That tubercle bacilli killed by heat have, nevertheless, been shown so frequently to retain immunizing ability thus provides a strong basis for confidence in their protective potency and stability, and should serve as a stimulus for the further exploration of methods which would appear *a priori* more suitable for the production of a vaccine.

Discussion

It is evident from this perusal of the literature that the majority of reports based on experiments in which nonliving antituberculosis vaccines were tested with some variations in the dosage, vehicle, and route of administration, method of killing, vaccination-challenge interval, and other experimental parameters, support the view that such vaccines possess approximately equal immunogenicity as living, attenuated ones. Although a number of technical problems require further attention, in the direction of combining the experiences of the many different groups of investigators in this field toward the development of *the most* effective nonliving preparation for use in man, there appears to be no reasonable ground for doubting in principle the immunizing capacity of killed tubercle bacilli and of some of their extracts, nor the complete safety of many such preparations. The largest question remaining in the field of nonliving vaccines against tuberculosis is, instead, why they have been so frequently, and often decisively, condemned as ineffective. A clear answer to this curious and frustrating paradox does not present itself, but an attempt will be made at least to delineate the circumstances which appear most responsible for its growth and perpetuation.

The first of all vaccines, living vaccinia virus,—and to this day the most effective—was already discovered almost a hundred years before the first attempts to immunize against tuberculosis were initiated. Empirical though Jenner's initial observations were, they nevertheless provided an experimental basis for vaccination attempts with either naturally or experimentally "attenuated" strains of other microorganisms. It is not surprising, therefore, that the recognition of the microbial nature of a disease was usually followed very shortly by vaccination attempts with living, attenuated vaccines, e.g., anthrax, cholera, chicken cholera, swine erysipelas, rabies, contagious abortion of cattle. It soon became apparent that a complete loss of infectivity of a microbial strain was usually also accompanied by a loss of immunizing potential (262). This observation gave rise to the belief that only a living organism still capable of initiating a disease process, albeit a relatively mild one, could evoke a significant degree of immunity to reinfection, a belief strengthened by the clinical truism that survivors of many infectious diseases develop a measurable degree of specifically acquired immunity. Opposed to this view stood the experimental findings of Koch, Straus, Prudden (*see* 17, 19, and 20), and others, that killed microorganisms, especially tubercle bacilli, could, when administered in sufficient quantities, produce a number of pathologic changes in the tissues of animals indistinguishable from those caused by the living bacilli, suggesting that immunity, too, could be elicited by nonliving organisms.

In the case of tuberculosis, nonliving preparations were therefore actually among the first vaccines tried but, although they were also among the first to be found effective, the bias in favor of a living vaccine was not dispelled. As has been pointed out by Long (*see* 118), "the whole story of immunization has led investigators to expect a solution of tuberculosis immunity through the use of a living virus," and the discovery of the vaccinating abilities of living BCG was greeted as corroboration of a long-felt truth, despite the considerable number of reports which

had already appeared by 1925 attesting to the efficacy of nonliving vaccines. Dead vaccines were conceded by some to have "certain favorable effects upon resistance to severe infections" (*see* 30) but, as they were thought capable, at best, of only approximating living ones, it was generally deemed futile to pursue their development further.

It must be admitted that the negative attitude toward nonliving vaccines against tuberculosis is not only rooted in biophilosophic considerations, but also has a certain basis in the failure of some early investigators to demonstrate their efficacy in experiments in which parallel series of animals vaccinated with living vaccines *did* exhibit a state of heightened resistance. These failures are hardly astounding, however. Very often, when nonliving vaccines have been compared with living ones, the methods and procedures of vaccination have been similar; both types of vaccine would be administered by the same routes, in identical vehicles, and in the same quantity; when the dose of a nonliving preparation *has* been varied, it has usually been in 2 to 100 multiples of the standard dose of living vaccine.

Although this approach seems to speak for good experimental design, it ignores the basic difference in the mode of action of living and nonviable vaccines. The activity of the former is usually based on the elaboration by the *living* microorganisms of an immunogenically adequate quantity of immunizing antigens *in vivo*, while that of the latter depends, perforce, on the *initial* introduction of an adequate quantity of immunogen. While even a very small number of living vaccine bacilli may eventually synthesize enough protective material to elicit some degree of immunity, ten-, a hundred-, or an even greater-fold amount of dead, nonreproducible vaccine may not be sufficient. On the other hand, the administration of too large a quantity of dead vaccine at one time may bring about severe toxic reactions which can nullify any potential immunogenic effect.

There are other factors which must be given special consideration in working with a nonliving vaccine, such as administration in an excipient, and by a route, which will guarantee its longest presence in sites found empirically to be most favorable for the development of immunity. It may thus be said that protective immunity is less difficult to achieve with a living vaccine, but only in the sense that this may be done with a minimum of experimental sophistication; it is, in fact, surprising and very encouraging that nonliving ones have so often been shown to be equally effective despite the frequently unfavorable circumstances of their administration. No evidence has yet been advanced, however, that living antituberculosis vaccines possess *inherent* biologic, or even technical, superiority. If anything, it is *living* vaccines which are inherently less desirable, because their most effective preparations must possess a considerable degree of invasiveness to permit their survival and growth in living tissue long enough to produce an effective quantity of antigen; they are thus, by nature, hazardous in direct proportion to their efficacy.

The protective activity of nonliving vaccines against diseases other than tuberculosis has never been challenged as widely or emphatically. This is not surprising. Even very small quantities of many preparations of killed bacilli stimulate the production of circulating antibodies and tissue hypersensitivity. Some protection against infectious diseases, immunity to which is based on antibody, can therefore be effected by any vaccine capable of eliciting an antibody response. The mechanism of immunity to tuberculosis, on the other hand, is obscure, and does not appear to be directly based on the production of classical antibody. At any rate, bacillary preparations which cause the production of high titers of circulating antibodies do not necessarily possess protective powers, and a more subtle, not yet recognized property is required of an effective vaccine. The large number of communications reporting the immunizing activity against tuberculosis of many different nonliving preparations of tubercle bacilli show that it is not difficult to obtain, empirically, a vaccine endowed with the necessary immunogenic properties. Nevertheless, the frequent observation that minor variations in preparatory procedure can destroy immunogenicity without affecting the vaccine's capacity to initiate antibody production also indicates that the usual criteria of immunologic activity have little bearing on the immunogenic efficacy of antituberculosis vaccines.

Still other circumstances have acted to keep alive the early conviction of the uselessness of nonliving vaccines in the case of tuberculosis. The ubiquity of the disease, the lack of effective therapy, and its special virulence among the

young, stimulated the most urgent search for both cure and prophylaxis, with an emphasis on cure, after the discovery of the tubercle bacillus. (In discussing the prophylactic implications of the Koch phenomenon, Römer wrote in 1910 (*see* 43) that "this result of Koch's experiment received much less attention than the retardation of an already established tuberculous infection which, according to Koch, is brought about by the reinfecting tubercle bacilli in the guinea pig."[12] Consequently, most "vaccine" preparations were tested for therapeutic as well as for prophylactic activity, and the therapeutic failure of a preparation often doomed its further study even when initial experiments revealed that it had definite prophylactic powers. Thus, for example, the considerable immunogenic activity found by Koch to reside in certain "tuberculins," or bacillary fractions (*see* 17), has remained largely ignored and unexploited for decades following the sharp disappointment in the failure of tuberculin to live up to the high *therapeutic* action promised by Koch in announcing its discovery. Other promising vaccines have been abandoned prematurely for similar reasons.

The urgency of the tuberculosis problem in the last decades of the nineteenth century and the early years of the twentieth had other unfortunate results for the study and development of nonliving vaccines. The rapid, and sometimes dramatic, development of vaccine prophylaxis and therapy against other infectious diseases led to the hope of finding early a *completely* effective antigen against tuberculosis as well, with the corollary result that any preparation which bestowed only limited resistance on healthy—or limited therapeutic effects on already diseased—individuals was impatiently dismissed. Even though living vaccines, too, failed to show the hoped-for activity, they were subjected to much more extensive study, always with the hope ahead that their similarity to fully virulent strains would one day result in the discovery of a preparation capable of bestowing that high degree of immunity then held by Marfan and many others to result from frank tuberculous disease in childhood. The possibility that the high immunity exhibited by survivors of certain forms of childhood tuberculosis might in large part reflect only a natural selection of persons endowed with greater natural resistance was usually not taken into consideration. The persistent failure by responsible investigators to find a fully effective vaccine against tuberculosis prepared the ground, moreover, for a wide receptiveness to exaggerated and often undocumented claims. More often than not, such claims were made for nonliving preparations, because these had at least the virtue of not doing too much harm. The invariable debacle with which these meteoric "vaccines" and remedies eventually met did little to dispel the general misgivings regarding nonliving antituberculosis preparations.

Another important reason for the negative evaluation of nonliving antituberculosis vaccines during the past thirty years has been a technical one. Experiments in tuberculosis vaccination are long and expensive, and few laboratories in the world possess adequate facilities to house the large numbers of animals necessary to test the variety of experimental conditions of vaccination necessary to establish the parameters of activity of a nonliving preparation. Because they show activity under a wider range of experimental circumstances, laboratory trials of living vaccines appear to be simpler and more rewarding. Especially since the advent of BCG, funds and space have therefore been allocated preferentially for the testing of living vaccines. In consequence, killed preparations have often been tested on a "hit-or-miss" basis, without the necessary variation of experimental factors and without the numbers of animals necessary to lend statistical significance to the results. Opposed to the statistical respectability of BCG, the evidence accumulated from studies on nonliving preparations—no matter how effective the majority has indicated them to be—could not dislodge the historic opinion of the superiority of living vaccines. Several large, and generally very well designed, experiments comparing the immunizing efficacy of living BCG and killed bacillary preparations have been conducted under WHO auspices during recent years; but, unfortunately, the largest quantity of killed vaccine employed in these experiments may well have been suboptimal, and the results therefore again "demonstrated" the inferiority of nonliving vaccines (*see* 244–246; *see also* 235).

[12] "Dieses Ergebnis des Kochschen Versuches viel weniger beachtet worden als die Rückwirkung, welche nach Koch die reinfizierenden Tuberkelbazillen auf die bereits vorhandene Tuberkeloseinfektion des Meerschweinchens haben soll."

A reinvestigation of the immunizing propensities of nonliving vaccines against tuberculosis, employing statistically significant numbers of animals and varying as widely as possible the experimental conditions of vaccination, has thus long been called for. Such an investigation was begun in the laboratories of Dr. René J. Dubos at the Rockefeller Institute for Medical Research in 1952, employing mice as the experimental animals (*see* 230). During the past three years, this work has been extended by the present writer to guinea pigs in the laboratories of the late Dr. A. Q. Wells at the Sir William Dunn School of Pathology at Oxford, and at the Microbiological Research Establishment in Porton, England. Some of these results, based on experiments involving more than 1,800 animals, have already been summarized briefly (*see* 8, 236); a detailed report will be made in succeeding communications (*see* 237, 238).

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CARDIOPULMONARY FUNCTION IN CHRONIC OBSTRUCTIVE EMPHYSEMA[1, 2, 3]

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INTRODUCTION

A number of reports have pointed out that chronic pulmonary emphysema is a disease which is characterized, pathophysiologically, by airway obstruction, increased residual volume of the lung, impaired mixing of gases in the lung, hypoxemia, carbon dioxide retention, pulmonary hypertension, and reduction of the diffusing capacity of the lung (1–5). The degree to which these various abnormalities are present, particularly during muscular exercises, is not entirely clear. This report presents the results of ventilatory studies and measurements of pulmonary vascular pressures and flows, of diffusion of gases in the lungs, and of arterial blood gas composition during rest and exercise in 22 cases of chronic pulmonary emphysema.

MATERIAL AND METHODS

The patients were all males with definite clinical diagnoses of emphysema (table 1). They all had severe shortness of breath on exertion and a variable degree of chronic cough, fluoroscopic evidence of impaired diaphragmatic function, and physical signs of emphysema. Patients with tuberculosis, bronchiectasis, or with a history of bronchial asthma which antedated the onset of shortness of breath were excluded from this study. In general, the patients had a relatively severe form of emphysema.

Ventilatory function was assessed from a spirometric tracing, the largest of at least two expirations being used for analysis on each occasion. Vital capacity and maximal midexpiratory flow rate (MMF) (6) were calculated from this tracing. Vital capacity was expressed as percentage of normal from a predicted value of 2.5 times the body surface area. Lung volumes were measured by the open-circuit method, using a nitrogen meter (7). Arterial blood gas composition was measured by the Van Slyke manometric technique for oxygen and carbon dioxide contents, pH was measured on a Cambridge pH meter, and the carbon dioxide tension ($P_{A_{CO_2}}$) was calculated from a nomogram. Analysis of arterial oxygen tension during oxygen breathing was performed with the Clark oxygen electrode using a system designed by Kreuzer and associates (8). For estimation of right-to-left shunts, the patient breathed oxygen for ten minutes, and the alveolar gas tension was assumed to equal the inspired oxygen tension minus an assumed 1 per cent alveolar nitrogen concentration minus the alveolar carbon dioxide tension. The difference between this alveolar oxygen tension and the arterial oxygen tension was used to calculate the right-to-left shunt. Diffusing capacity for carbon monoxide (D_{CO}) was measured by the steady-state method (9). Pulmonary vascular pressures and flows were measured by passing a polyethylene (10) or conventional nylon catheter into the pulmonary artery for recording of pressures, and cardiac output was calculated from the Fick formula. Total pulmonary resistance (TPR) was calculated by dividing the mean pulmonary arterial pressure by the cardiac output. Exercise studies were conducted in the supine position, the patient pedaling against a fixed resistance at the maximal rate that he could sustain for five minutes. Blood and gas collections were obtained during the last two minutes of exercise. Lung dead space (V_D) was calculated from the Bohr equation using the arterial plasma carbon dioxide (P_{CO_2}) as equivalent to the alveolar P_{CO_2}, and the result was expressed as a fraction of the tidal volume (V_D/V_T).

Each patient was seen repeatedly for measurement of ventilatory function. Arterial blood studies were performed less frequently, in conjunction with measurements of diffusing capacity, and cardiac catheterization was performed at least once in all but one patient. Only data obtained on ambulatory patients free of acute infection or of clinical evidence of right heart failure are reported.

RESULTS

In table 2 may be seen the data for ventilatory function, resting diffusing capacity, and arterial blood gas composition in these 22 patients. In every instance there was severe expiratory delay, as evidenced by reduction of the maximal mid-

[1] From the Cardiorespiratory Laboratory, Grasslands Hospital, Valhalla, New York.

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[3] Presented in part before the Medical Session, as part of *Section 1B*, at the annual meeting of the American Trudeau Society, Chicago, Illinois, May 25, 1959.

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TABLE 1
EMPHYSEMA

Patient	Symptoms
ORS	5 years of progressive dyspnea; 1 block limit; chronic cough.
BOR	10 years of variable dyspnea (1–3 flights-of-stairs limit) with episodic nonproductive cough and wheeze.
TEB	30 years of bronchitis; pneumonia 8 times; 13 years of dyspnea, cough, wheeze, worse in winter; severe for 3 years.
CUL	5 years of slightly productive cough, 3 years of progressive dyspnea; incapacitated.
JOH	Dyspnea with colds for 8 years, progressive dyspnea for 8 months, now bothered by 1 flight; no cough.
UBB	6 years of severe dyspnea and cough since attack of pneumonia; limited to slow walk on level.
DOR	5 years of progressive dyspnea and slight cough since pneumonia; 1 flight limit.
LAN	6 years of progressive shortness of breath, incapacitated for 2 years; chronic, heavily productive cough.
ECK	Onset of dyspnea 15 years ago; static for 12 years; 1 flight limit; mild cough productive of thick sputum.
MAU	Gradually progressive dyspnea for 3 years; improved when stopped smoking and now can climb 4 flights; mild chronic cough.
HOZ	20 years of productive cough; dyspnea for 5 years, now limited to 2 blocks.
HUN	10 years of variable dyspnea and cough; now short of breath on slight exertion.
FOR	10 years of shortness of breath and cough; repeated attacks of bronchitis; currently able to work.
MEC	8 years of dyspnea and productive cough, after pneumonia; incapacitated.
CON	Gradually progressive shortness of breath with mild cough for 10 years, now limited to 1 flight.
AND	10 years of dyspnea and wheeze relieved by bronchodilator; 15 years of cough; now limited to 2 flights.
DeP	5 years of dyspnea on stairs and dry cough; sputum and 3-block limit for a few months; can work.
DUG	15 years of dyspnea, wheeze, and morning cough, recently noticeable on a few yards of level walking; frequent respiratory infections.
MAR	10 years of progressive dyspnea, limited to ½ block; 4–8 ounces thick sputum per day.
COS	Progressive dyspnea and chronic cough for 5 years, now limited to 2 flights.
BUC	8 years of progressive productive cough, severely limited.
CRU	10 years of moderate dyspnea, occasional cough; can climb 2 flights.

All patients were moderate-to-heavy cigarette smokers of long-standing duration.

expiratory flow rate, which was never above 1 liter per second in 266 observations. The vital capacity was reduced in each subject, as was the diffusing capacity. The arterial oxygen saturation was always subnormal, varying from 80 to 91 per cent, and there was a slightly smaller degree of carbon dioxide retention.

Exercise was performed one to four times by 19 patients and resulted in a decrease of the oxygen saturation from −12 to + 4 per cent (average: −3.4 per cent), and an increase of the P_{CO_2} from −3 to + 14 mm. of mercury (mean: + 2.4 mm. of mercury). The diffusing capacity increased from a mean of 7.1 at rest to 9.2 during exercise, with a range of −3.8 to + 5.9 cc. per minute per mm. of mercury.

The results of measurement of the arterial oxygen tension ($P_{a_{O2}}$) during oxygen breathing are shown in table 3. Assuming an arteriovenous oxygen difference of 4.60 volumes per cent, the average value found in twenty-four measurements in these patients at rest, the percentage of right-to-left shunt was calculated and ranged from 0 to 15 per cent of the cardiac output. In 12 normal subjects, the right-to-left shunt was found to be 2.9 ± 2.4 per cent. The magnitude of this shunt varied with the degree of hypoxemia, but was not related to the level of the diffusing capacity. Without exception, the shunt was far too small to explain the degree of hypoxemia present.

Lung volumes were measured in 15 patients, and the average value of residual volume/total lung capacity was 65 per cent (range: 55–74 per cent). The seven-minute nitrogen was 6 per cent, ranging from 2 to 10 per cent. In 20 patients,

TABLE 2

DATA ON VENTILATORY FUNCTION, RESTING DIFFUSING CAPACITY, AND ARTERIAL BLOOD GAS COMPOSITION IN 22 CASES OF PULMONARY EMPHYSEMA

Patient	Age	BSA M^2*	MMF L./Sec.†		N‡	Mean V.C.§	D_{CO}‖		N‡	Arterial Oxygen Saturation		N‡	Arterial P_{CO_2} in Mm. Hg		N‡	Mean Hct.¶
			Mean	Range			Mean	Range		Mean	Range		Mean	Range		
						(per cent)				(per cent)						(per cent)
ORS	62	1.78	0.42	0.37–0.59	8	57	9.4		1	86	84–88	2	57	56–58	2	52
BOR	65	1.62	0.51	0.38–0.71	6	76	6.1		1	91	90–92	2	38	37–41	3	41
TEB	64	1.60	0.45	0.36–0.68	13	55	5.8		1	83	79–88	4	63	52–72	3	50
CUL	59	1.84	0.33	0.26–0.51	9	55	6.3	5.1–7.4	2	88	85–91	2	56	54–58	2	54
JOH	51	1.89	0.27	0.19–0.39	16	46	5.7		1	84	82–88	5	69	56–78	6	49
UBB	64	1.77	0.46	0.36–0.70	23	54	3.2	2.3–4.6	5	91	80–93	10	48	40–56	9	45
DOR	62	1.97	0.40	0.37–0.55	5	47	6.8	4.9–8.8	2	85	80–89	2	56	50–61	2	54
LAN	62	1.80	0.36	0.24–0.50	32	53	5.3	4.2–7.2	3	80	66–87	10	57	44–63	9	44
ECK	78	1.54	0.32	0.16–0.63	28	57	3.3	2.5–4.0	2	89	83–95	7	46	41–50	5	42
MAU	58	1.71	0.33	0.32–0.34	3	42	4.9		1	81		1	51		1	51
HOL	62	1.96	0.31	0.22–0.39	22	38	8.7	5.8–10.1	3	81	76–88	5	59	57–64	7	49
HUN	59	1.50	0.31	0.24–0.60	14	64	8.9	6.8–11.5	3	91	90–91	3	41	38–47	3	41
FOR	66	1.84	0.35	0.26–0.52	11	72	6.5		1	84	83–85	2	49	48–50	2	45
MEC	72	1.38	0.23	0.16–0.28	9	44	3.4		1	84	76–89	5	45	38–50	5	44
CON	56	1.77	0.36	0.27–0.43	5	52	13.4		1	76		1	56		1	53
AND	69	1.79	0.56	0.34–0.71	14	49	10.5	9.5–11.6	2	88	82–91	3	45	42–49	3	43
DeP	55	1.74	0.47	0.33–0.65	6	63	4.9	3.3–6.8	3	88	86–89	3	44	42–50	3	47
DUG	52	1.76	0.45	0.27–0.69	13	55	6.8	5.6–8.0	2	88	84–92	4	45	40–52	4	51
MAR	62	1.74	0.39	0.21–0.50	19	54	9.5	8.2–10.4	3	90	88–93	5	46	40–50	6	41
COS	68	1.66	0.82	0.57–1.0	3	54	6.7		1	90		1	37		1	44
BUC	79	1.61	0.35	0.28–0.43	2	44	12.0		1	87		1	58		1	42
CRU	78	1.88	0.69	0.64–0.80	5	51	9.1	8.5–9.0	2	88	84–91	3	47	45–48	3	44
Mean	64		0.42			54	7.1			86			51			47

* BSA M^2 = body surface area in square meters
† MMF L./Sec. = maximal midexpiratory flow rate in liters per second
‡ N = number of measurements
§ Mean V. C. = mean vital capacity
‖ D_{CO} = diffusing capacity for carbon monoxide in cc. per minute per mm. of mercury
¶ Mean Het. = mean hemotocrit

TABLE 3

MEASUREMENT (OBTAINED DURING OXYGEN BREATHING) OF ALVEOLAR OXYGEN TENSION ($P_{A_{O_2}}$), ARTERIAL OXYGEN TENSION ($P_{a_{O_2}}$), AND CALCULATED RIGHT-TO-LEFT SHUNT IN 9 PATIENTS WITH EMPHYSEMA

Patient	$P_{A_{O_2}}$ Mm. Hg	$P_{a_{O_2}}$ Mm. Hg	R-L Shunt	D_{CO}*	$S_{a_{O_2}}$†
			(per cent)		(per cent)
UBB	623	626	0	2.7	90
DOR	639	617	1	4.9	89
AND	636	404	13	9.5	82
JOH	609	349	15	5.7	70
ECK	629	591	2	4.0	89
HUN	632	529	6	11.5	90
UBB	621	545	5	3.3	84
HOL	605	523	5	1.6	87
CRU	632	572	4	9.6	88

*D_{CO} = resting diffusing capacity in cc. per minute per mm. of mercury
† $S_{a_{O_2}}$ = arterial oxygen saturation

the average value for lung dead space/tidal volume was 49 per cent, ranging from 33 to 66 per cent.

In table 4 are listed the data obtained on these patients when diffusing capacity and pulmonary vascular pressures and flows were measured simultaneously both at rest and during exercise. Resting pulmonary hypertension was present in all but 3 patients and the resting cardiac index was extremely variable, the average value of 3.1 liters per minute per square meter being within the normal range. Exercise resulted in abnormal increase of the pulmonary arterial pressure in all but 2 patients, increase of the diffusing capacity in all but one patient, and increase of the cardiac output in all but one patient.

DISCUSSION

From these results, the pathophysiology of chronic pulmonary emphysema emerges as a

TABLE 4

SIMULTANEOUS MEASUREMENTS OF ARTERIAL CARBON DIOXIDE TENSION ($P_{a_{CO_2}}$), ARTERIAL OXYGEN SATURATION ($S_{a_{O_2}}$), OXYGEN CONSUMPTION (V_{O_2}), DIFFUSING CAPACITY (D_{CO}), PULMONARY ARTERIAL PRESSURE AND FLOW (PAP AND F), AND LUNG DEAD SPACE/TIDAL VOLUME (V_d/V_t)

Patient	State	$P_{a_{CO_2}}$ Mm. Hg	$S_{a_{O_2}}$	V_{O_2} Cc/Min.	D_{CO}, Cc/Min Mm. Hg	PAP and F, Mm. Hg			Cardiac Index, L./Min./M²	VD/VT
						Systolic	Diastolic	Mean		
			(per cent)							(per cent)
COS	R*	37	90	222	6.7	32	11	19	3.23	39
	E†	40	87	486	9.2	47	21	29	4.65	39
BUC	R	58	87	230	12.0	53	21	33	4.45	40
	E	56	85	397	12.7	67	32	45	5.17	44
CRU	R	45	84	260	8.5	26	8	15		57
	E	44	90	566	9.1	49	20	32	4.22	56
DEP	R	40	86	227	6.8	29	11	20	2.72	56
	E	43	87	546	7.2	41	19	24	4.26	51
	R	42	89	204	3.3	27	9	18	2.50	66
	E	49	89	605	7.6	66	24	40	3.88	54
AND	R	43	82	292	9.5	22	5	12	3.77	43
	E	42	81	571	12.4	19	5	12	4.62	41
CON	R	56	76	246	13.4	37			3.87	53
	E	58	77	728	10.3	72			5.12	46
MECH	R	40	89	208	3.4	40	19	29	3.63	
	E	54	89	306		54	27	38	3.65	
FOR	R	49	84	227	6.5	30			2.57	46
HUN	R	41	91	195	8.3	35			2.20	43
HOL	R	58	85	355		32	22	27	4.00	
	E		81	641				52	4.56	
	R	58	82	227	5.8	48	13	26	3.15	58
	E	64	80	371	7.6	64	28	44	6.02	57
MAU	R	51	81	263	4.9	32	13	20	2.03	46
	E	54	69	448	5.7	59	24	38	3.62	45
DOR	R	50	89	283	4.9	36	13	24	4.17	52
	E	52	86	462	10.8	60	23	38	3.52	44
UBB	R	42	80	219	3.2	47	20	30	2.40	45
	E	43	78	371	7.2	47	20	31	2 70	42
CULL	R	58	85	250	7.4	39	18	25	2.40	52
BOR	R	35	89	265	6.1	25	12	17	3.23	33
	E	37	92	602	8.2	45	24	35		33

* R = at rest
† E = during exercise

relatively homogeneous picture of chronic, severe airway obstruction, mild hypoxemia, and carbon dioxide retention slightly exaggerated by exercise, pulmonary hypertension particularly apparent during exercise, increased residual volume of the lung, and severe reduction of the diffusing capacity during rest and exercise. Some of these abnormalities will be discussed individually with particular reference to their reflection of the structural abnormalities present in pulmonary ephysema.

The diffusing capacity for carbon monoxide (D_{CO}) was reduced at rest and during exercise in all of the patients described in this report. The question arises whether the low diffusing capacity resulted from a true diffusion limitation due to loss of alveolar capillary surface or from some other abnormality of pulmonary function.

Bates has recently discussed the technical problems encountered in measurement of the diffusing capacity, particularly in pulmonary emphysema, and has pointed out that the steady-state carbon monoxide method theoretically may give a falsely high value (11). However, Forster has noted that nonuniform ventilation in respect to either diffusion or circulation may lead to a low diffusing capacity as measured by the steady-state technique (12). McNeill, Rankin.

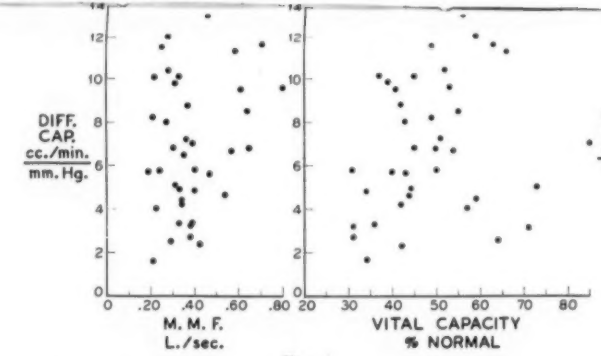

FIG. 1

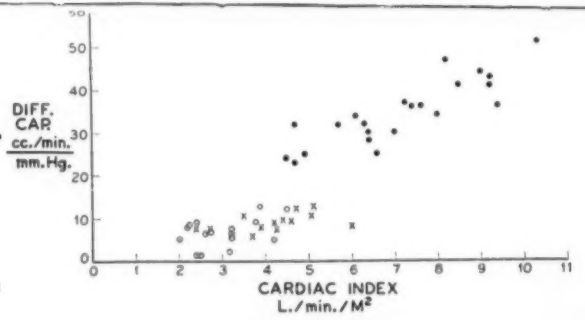

FIG. 2

FIG. 1 (Left). Measurements, obtained on the same day, of the resting diffusing capacity, vital capacity, and maximal midexpiratory flow rate (MMF) in 22 patients with chronic pulmonary emphysema. FIG. 2 (Right). Simultaneous measurements of diffusing capacity and cardiac index at rest (○) and during muscular exercise (×) in chronic pulmonary emphysema. Normal data obtained by Shepard and associates (27) are shown for comparison (●).

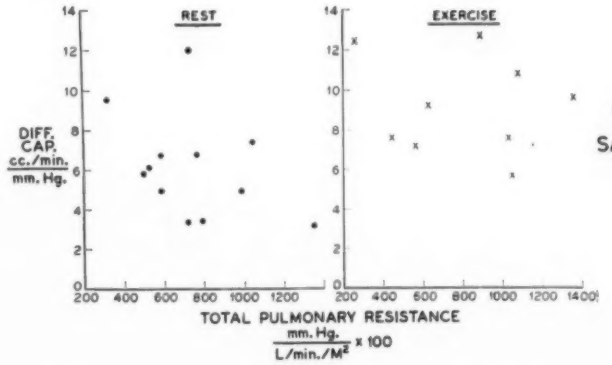

FIG. 3

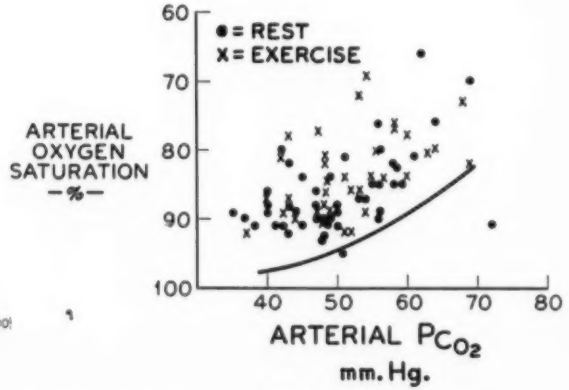

FIG. 4

FIG. 3 (Left). Relationship between diffusing capacity and total pulmonary resistance to flow at rest and during exercise. FIG. 4 (Right). Simultaneous measurements of resting arterial oxygen saturation and arterial carbon dioxide tension.

and Forster are of the opinion that a low steady-state diffusing capacity measurement in pulmonary emphysema may result from impaired mixing of gases in the lung and altered ventilation to perfusion ratios. They cite as evidence for this the observation that the diffusing capacity measured by the single-breath technique is not necessarily reduced (13). This tenet is open to question, however, since the single-breath method may lead to falsely low values if ventilation/diffusion ratios are nonuniform throughout the lung, and if the volume of the lung into which carbon monoxide is inspired differs from that previously measured by a non–single-breath technique. In addition, this hypothesis implies that the steady-state method is sufficiently influenced by impaired mixing of gases in the lung to yield a low diffusing capacity in emphysema on this basis alone. Against this hypothesis is the observation that patients with asthma studied in this laboratory had evidence of increased residual volume and severe impairment of mixing of gases in the lung, yet a normal steady-state carbon monoxide diffusing capacity. Bates has also pointed out that the diffusing capacity may be normal in asthma (14).

Severe airway obstruction is not necessarily associated with a low diffusing capacity since, as mentioned above, patients with bronchial asthma may have a normal diffusing capacity. As shown in figure 1, there was no relationship between the degree of reduction in maximal midexpiratory flow rate or vital capacity and the diffusing capacity in the emphysematous patients reported in the present study. This observation supports the findings of Shepard and his coworkers that the reduction of the diffusing capacity in chronic obstructive airway disease is not correlated with the degree of airway obstruction, and adds weight to the suggestion that loss of alveolar-capillary surface and airway obstruction are independent abnormalities in emphysema (15).

That the low diffusing capacity is not due to diminished blood flow is evident from the fact that the average cardiac index in these patients was normal and, as seen in figure 2, the diffusing capacity during exercise was reduced for the degree of blood flow obtained.

If the diffusing capacity is a reflection of alveolar-capillary surface area, it should bear some relationship to the resistance to blood flow through the lung. The pulmonary vascular resistance cannot be calculated with accuracy in the absence of measurement of the left atrial pressure, but the total pulmonary resistance was calculated in those patients in whom cardiac output and mean pulmonary arterial pressure were known by dividing the mean pulmonary arterial pressure by the cardiac output. As seen in figure 3, there appeared to be a relationship between the two functions, but it was not statistically significant.

Whether the low diffusing capacity in emphysema actually reflects loss of alveolar-capillary

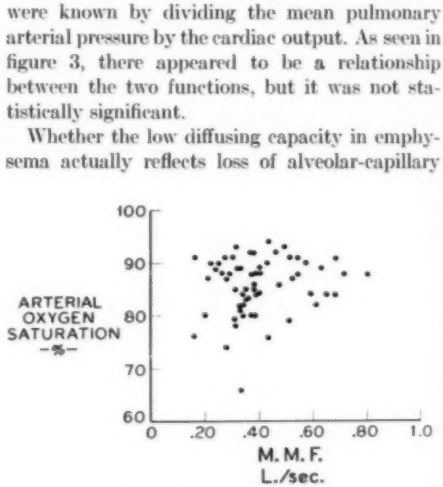

FIG. 5. Relationship between arterial oxygen saturation and maximal midexpiratory flow rate (M.M.F.).

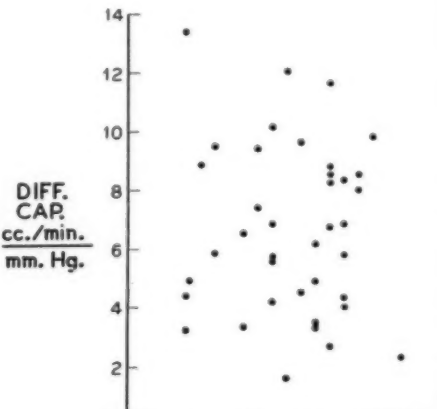

FIG. 6. Simultaneous measurements of diffusing capacity and arterial oxygen saturation at rest.

surface or inequality of ventilation and perfusion and/or diffusion in the lung cannot be stated with assurance at this time. Nevertheless, a low steady-state carbon monoxide diffusing capacity is a distinctive of chronic obstructive emphysema.

Hypoxemia might result from diminished alveolar ventilation, from impaired diffusion of oxygen across the alveolar membrane, from a right-to-left shunt, or from inequality of ventilation and perfusion throughout various parts of the lung.

In figure 4, the arterial oxygen saturation in these patients is plotted against the simultaneously measured arterial carbon dioxide tension. The solid line represents the theoretic value for arterial oxygen saturation and arterial carbon dioxide tension which would be present in patients in whom diminished alveolar ventilation were the only abnormality present (16). It may be seen that, almost without exception, the degree of hypoxemia is greater than the degree of carbon dioxide retention, suggesting that inadequate alveolar ventilation is not the primary abnormality. In figure 5, the arterial oxygen saturation is plotted against the maximal midexpiratory flow rate, and the correlation between hypoxemia and expiratory delay is very poor, further evidence that the hypoxemia is not primarily due to reduced ventilation.

In figure 6 are shown the simultaneous data obtained for diffusing capacity and arterial oxygen saturation and, again, there is no correlation between hypoxemia and reduction of diffusing capacity. Furthermore, if the hypoxemia was the result of impaired diffusion, it should increase markedly during exercise; this was not the case in this study. In addition, theoretic calculations by Shepard (17) and by Williams (16) have shown that the diffusing capacity must be lower than was found in these patients, both during rest and exercise, before significant hypoxemia would appear.

A true right-to-left shunt may be excluded as the cause of hypoxemia in emphysema by the data shown in table 3. Although these patients did show evidence of a large right-to-left shunt through the lungs during oxygen breathing, this was never sufficient to account for the degree of hypoxemia present during air breathing. Similar results have been reported by others (18).

The degree of shunt did correlate with the degree of hypoxemia present during air breathing, but this is compatible with the assumption that the hypoxemia was actually the result of disordered ventilation/perfusion relationships in the lung, representing one ultimate in such disorder—perfusion of totally unventilated alveoli. Some of the apparent correlation between the degree of shunt and hypoxemia may be the result of assuming an alveolar nitrogen concentration of 1 per cent after ten minutes of oxygen breathing for calculation of the right-to-left shunt. In the presence of severe airway obstruction, the alveolar nitrogen concentration may actually be higher than 1 per cent, so that the alveolar oxygen tension difference might have been smaller than was calculated.

A number of writers have emphasized that pulmonary hypertension in emphysema correlates with the degree of hypoxemia present (19–22). Such a correlation is evident when data obtained on patients with acute pulmonary insufficiency

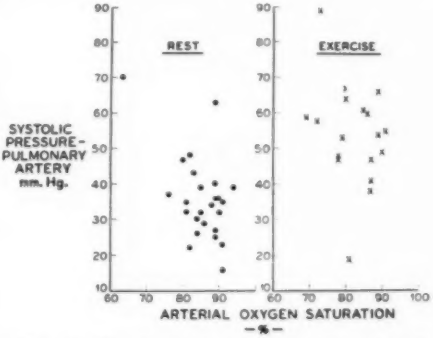

FIG. 7. Simultaneous measurements of pulmonary arterial pressure and arterial oxygen saturation at rest and during exercise.

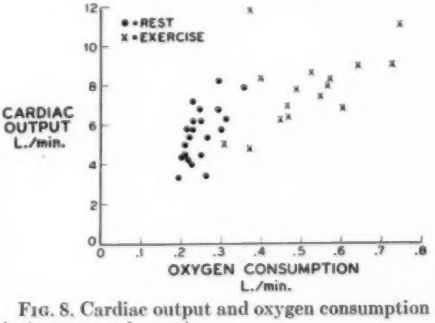

FIG. 8. Cardiac output and oxygen consumption during rest and exercise.

and severe hypoxemia are examined, but, as may be seen in figure 7, this relationship is not so clear in the ambulatory patient free of acute pulmonary insufficiency and/or right heart failure. As suggested by others, the mild resting pulmonary hypertension in emphysema is probably the result of reduction of the pulmonary vascular bed, and severe pulmonary hypertension appears only when the primary and secondary effects of hypoxemia are superimposed. The brisk increase of pressure in the pulmonary artery during exercise is to be expected when cardiac output increases through a restricted, nonexpansile pulmonary vascular bed.

In contrast to early reports of an increased cardiac output in pulmonary emphysema, recent writers have indicated that the resting cardiac index may be normal in this disease (20, 21). In twenty studies of the resting cardiac output in the patients reported above, the average value was 3.11 liters per minute per square meter of body surface area (range: 2.03 to 4.45). During exercise (figure 8), the cardiac index increased normally in 2 patients. The remaining patients had exercise cardiac indices lower than that reported as normal by Asmussen and Nielsen (23), and 7 of 16 studies revealed a cardiac index below the 95 per cent confidence limit reported by Donald and his associates, (24). It seems likely that a normal cardiac index is apt to be present at rest in the ambulatory patient with emphysema, and the subnormal increase of cardiac output during exercise may be the result of coexistent left ventricular disease or of inadequate right ventricular function in the face of an increased pulmonary vascular resistance.

In these studies, an attempt was made to have the patients perform the maximal exercise which they could carry out for five minutes. The reason for their inability to do more severe exercise is not entirely clear from these or from other studies. The lack of severe hypoxemia or carbon dioxide retention during exercise indicates that abnormalities of the arterial blood gas composition were not a limiting factor. Although the cardiac output failed to rise normally during exercise, the arteriovenous oxygen difference was not as wide as that seen in patients who are limited by heart failure, and a circulatory limitation is unlikely. A reasonable assumption is that exercise intolerance results from the subjective discomfort of hyperventilation in the presence of severe airway obstruction, the work of breathing becoming intolerable at mild levels of exertion (25).

It should be pointed out that the steady-state exercise used in these studies differs from peak exercise which may be carried out for short

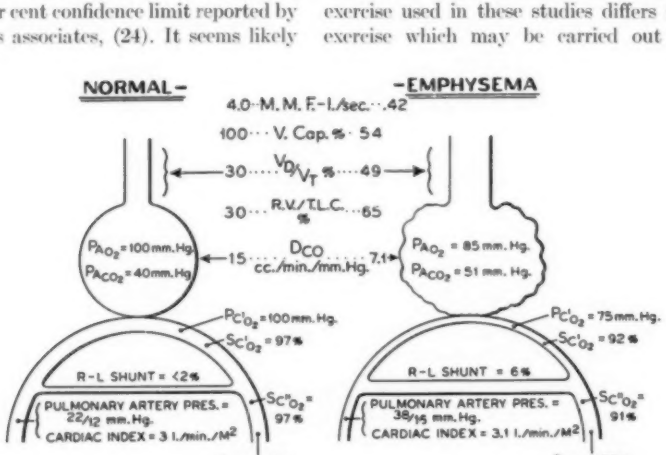

FIG. 9. Average resting data in chronic pulmonary emphysema as contrasted to the normal. The partial pressure of oxygen in the blood leaving the alveolar capillaries ($P'_{c_{O_2}}$) was calculated from knowledge of the diffusing capacity, oxygen consumption, and alveolar oxygen tension ($P_{a_{O_2}}$). The oxygen saturation of that blood ($S_{c'_{O_2}}$) was read from a standard oxygen disocciation curve. The oxygen saturation of that blood after mixture with the measured per cent R-L shunt ($S_{c''_{O_2}}$) was calculated from knowledge of the average arteriovenous oxygen difference.

periods of time. Under the latter conditions, the arterial oxygen saturation may fall precipitously and the arterial P_{CO_2} may rise when the alveolar ventilation fails to keep pace with the increased metabolic rate, no small part of which is due to the oxygen requirements of the respiratory muscles (26). It is of interest that most patients with emphysema will not carry out steady-state exercise which would result in severe hypoxemia and carbon dioxide retention.

The average resting data on these patients is summarized in schematic form in figure 9. The impaired ventilatory function, presumably largely the result of airway obstruction, is reflected in a very low maximal midexpiratory flow rate and in reduction of the vital capacity. The residual volume of the lung is considerably increased. The lung dead space is large, presumably as a result of both enlargement of the dimensions of the air-conducting passageways and of ventilation of poorly perfused alveoli. Moderate reduction of the alveolar ventilation is reflected in elevation of the alveolar carbon dioxide tension and in reduction of the alveolar oxygen tension. The partial pressure of oxygen in the blood leaving the alveolar capillaries is slightly reduced because of the low diffusing capacity. This blood becomes further reduced in oxygen content by admixture with shunted venous blood. The arterial oxygen saturation is even lower than this because of disordered ventilation/perfusion relationships in the lung. The cardiac output is normal, and there is slight resting pulmonary hypertension.

Although this represents the pathophysiology of severe pulmonary emphysema, the sequential development of this situation with respect to each aspect of pulmonary function awaits further study.

Summary

Repeated studies of cardiorespiratory function in 22 patients with chronic pulmonary emphysema have revealed the following: severe and persistent reduction of the maximal midexpiratory flow rate and vital capacity; consistent reduction of the steady-state carbon monoxide diffusing capacity during both rest and exercise; mild hypoxemia and carbon dioxide retention at rest, slightly increased by exercise; increased residual volume of the lungs with impaired mixing; increased lung dead space; pulmonary hypertension which was mild at rest but fairly severe during exercise; and a cardiac index which was normal at rest but subnormal during exercise.

The outstanding abnormalities of cardiopulmonary function were the persistent airway obstruction and the reduced diffusing capacity. The latter was interpreted as a reflection of the reduced alveolar-capillary surface area which is the pathologic criterion of emphysema. By calculating the effects of the reduced alveolar ventilation, low diffusing capacity, and measured right-to-left shunt in these patients, it was found that the major contribution to hypoxemia was the presence of disordered ventilation/perfusion relationships in the lung.

Sumario

La Función Cardiopulmonar en el Enfisema Oclusivo Crónico

Estudios repetidos de la función cardiorrespiratoria en 22 enfermos de enfisema pulmonar crónico han revelado lo siguiente: disminución intensa y persistente de la velocidad de la corriente mesoespiratoria máxima y de la capacidad vital; disminución constante de la capacidad difusora del monóxido de carbono en la fase estable durante tanto el descanso como el ejercicio; leve hipoxemia y retención del bióxido de carbono en descanso, acrecentadas ligeramente por el ejercicio; mayor volumen residual en los pulmones con deterioro de la mezcla; mayor espacio pulmonar inerte; hipertensión pulmonar, que era leve en descanso, pero bastante intensa durante el ejercicio; y un índice cardíaco que era normal en descanso, pero subnormal durante el ejercicio. Las anomalías destacadas de la función cardiopulmonar fueron la persistente oclusión de las vías aéreas y la reducida capacidad difusora. Esta última se tomó como reflejo de la reducida área alvéolo-capilar que es la pauta histopatológica del enfisema. Calculando el efecto de la reducida ventilación alveolar, la baja capacidad difusora y la desviación derecha-a-izquierda medida en estos enfermos, se descubrió que el mayor aporte a la hipoxemia procedía de la presencia de trastornos de la relaciones de la ventilación-perfusión en los pulmones.

Resume

La fonction cardio-pulmonaire dans l'emphysème obstructif chronique

Des études répétées de la fonction cardio-respiratoire effectuées chez 22 patients atteints d'emphysème obstructif chronique ont révélé les phénomènes suivants: une réduction importante et persistante du débit expiratoire moyen maximal et de la capacité vitale; une réduction de l'état

constant de la capacité de diffusion du monoxyde de carbone durant le repos et l'exercice; une hypoxie et une rétention du CO_2 au repos, qui est légèrement augmentée par l'exercice; un volume pulmonaire résiduel accru avec déficience du mélange gazeux; une augmentation de l'espace mort; une hypertension pulmonaire qui était légère au repos, mais assez marquée durant l'exercice; un indice cardiaque normal au repos, mais subnormal durant l'exercice. Les anomalies saillantes de la fonction cardio-pulmonaire consistaient en une obstruction permanente de la voie respiratoire et une réduction de la capacité de diffusion. Cette dernière détermination a été interprétée comme la résultante de la réduction de la surface alvéolo-capillaire, qui est le critère pathologique de l'emphysème. En calculant les effets de la ventilation alvéolaire réduite, de la capacité de diffusion peu élevée et de la mesure du shunt de droite à gauche chez ces malades, on a découvert que la contribution majeure à l'hypoxie était la présence d'une altération des rapports de la perfusion ventilatoire dans le poumon.

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CARDIOPULMONARY FUNCTION IN PULMONARY FIBROSIS[1, 2]

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INTRODUCTION

In 1951, Wright and Filley reviewed the ways in which pulmonary fibrosis may affect the functioning of the lungs (1). They pointed out that some patients with pulmonary fibrosis demonstrated little or no abnormality of pulmonary function. However, methods of assessing the integrity of the pulmonary vascular bed and the diffusion surface of the lung were not at their disposal. This report presents the ventilatory and diffusion data on 47 patients with pulmonary fibrosis, 12 of whom had detailed studies of the pulmonary circulation and arterial blood gas composition during rest and exercise.

METHODS

The patients were adults with roentgenographic evidence of pulmonary fibrosis. Diagnoses included tuberculosis, sarcoidosis, silicosis, and fibrosis of unknown etiology. Ventilatory function was assessed by measurement of the minute ventilation and calculation of the vital capacity, timed vital capacity, and maximal midexpiratory flow rate (MMF) (2) from a spirometric tracing. The diffusing capacity of the lung was measured by the steady-state carbon monoxide technique developed by Filley and associates (3) or by a modification of this technique (4). Arterial blood gas composition was determined by measurement of the pH on a pH meter and by Van Slyke manometric analysis for oxygen and carbon dioxide content. Cardiac catheterization for measurement of right heart pressures and cardiac output by the Fick procedure was carried out with a radiopaque nylon catheter or polyethylene tubing (5). Exercise studies were performed by having the supine patient pedal against a fixed resistance at a constant rate for five minutes, and blood samples and expired air collections were obtained during the last two minutes of exercise.

RESULTS

Seventy-two measurements of vital capacity and of exercise diffusing capacity were made in 47 patients with pulmonary fibrosis. The results are shown in figure 1. In only 3 instances were both measurements normal. In 4 instances the diffusing capacity was normal and the vital capacity was reduced. In 15 instances the vital capacity was normal and the diffusing capacity was reduced. In the majority of cases both measurements were subnormal.

In tables 1 and 2 the data are presented on 12 patients studied in detail. These patients demonstrated a variable degree of ventilatory impairment, a variable degree of carbon dioxide retention, and a variable elevation of pulmonary arterial pressure. All patients had reduction of the diffusing capacity during both rest and exercise, and hypoxemia at rest which was generally aggravated by exercise. The cardiac output was normal or elevated at rest and during exercise in every patient.

Various combinations of abnormalities are represented in this group. The patient *Gal* had normal ventilatory function, minimal abnormality of arterial blood gas composition, normal pulmonary arterial pressure during exercise, yet marked reduction of the diffusing capacity. The patient *Dau*, the only individual with active tuberculosis, had the most marked impairment of ventilatory function in the entire group, with severe hypoxemia and carbon dioxide retention and a low diffusing capacity; yet, the pulmonary arterial pressure was normal. Three other patients (*Bur*, *McK*, and *Whi*) with less severe impairment of ventilatory function and reduction of the diffusing capacity also had normal pulmonary arterial pressures even during muscular exercise. The 3 patients with severe pulmonary hypertension (*Who*, *Gar*, and *Spe*) had moderate to severe reduction of vital capacity, though not necessarily more reduction of the diffusing capacity or more serious alteration of arterial blood gas composition than did the other patients.

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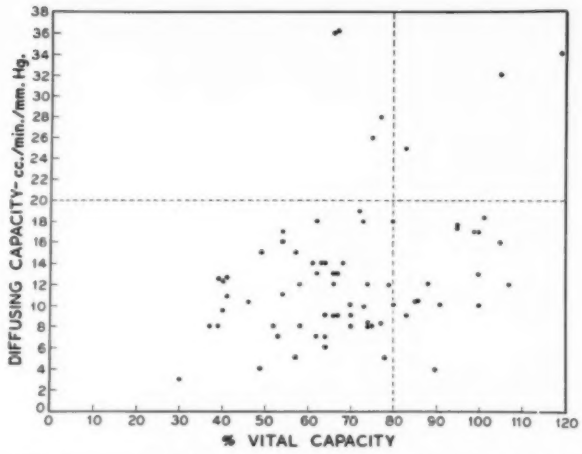

FIG. 1. Exercise diffusing capacity plotted against vital capacity in 47 cases of pulmonary fibrosis. The dotted lines represent the lower limits of normal for the two tests.

TABLE 1

IDENTIFYING DATA FOR TWELVE PATIENTS WITH ROENTGENOGRAPHIC EVIDENCE OF DIFFUSE PULMONARY FIBROSIS

Patient	Diagnosis	Age	Sex	B.S.A. M^2*	Clinical Data
Bur	Tuberculosis	33	M	1.92	Treated with chemotherapy and steroids 3 years ago; no recent symptoms.
McK	Sarcoidosis	39	F	1.62	Shortness of breath for 2 years; relieved by steroids.
Gal	Tuberculosis	47	M	2.00	Moderate shortness of breath for 4 years; dry cough, able to work.
Gut	Sarcoidosis	50	M	1.88	Pulmonary fibrosis on roentgenogram for 3 years; no symptoms.
Dau	Active tuberculosis	52	M	1.58	Extensive, active tuberculosis for 7 years.
Whi	Bronchogenic carcinoma, tuberculosis	45	F	1.69	Chronic bronchitis for many years; progressive dyspnea for one month.
Den	Tuberculosis	44	M	1.86	No symptoms; inactive tuberculosis.
Nic	Tuberculosis	37	M	1.78	Genitourinary tuberculosis with hematogenous spread to lungs; mild, intermittent dyspnea for 2 years.
Min	Silicosis	58	M	1.70	No symptoms, nodular fibrosis on roentgenogram.
Who	Silicosis	54	M	1.84	Dyspnea on heavy exertion for 3 months.
Gar	Sarcoidosis	41	F	1.80	3 years of chest pain and progressive shortness of breath; 1 block limit.
Spe	Sarcoidosis	28	M	1.64	7 years of progressive, severe dyspnea; completely incapacitated.

* B.S.A.M^2 = Body surface area in square meters.

DISCUSSION

These studies suggest that the outstanding abnormality of pulmonary function in pulmonary fibrosis is reduction of the diffusing capacity of the lung. The diffusing capacity was reduced more often than the vital capacity in the 47 patients on whom only these measurements were made. All 12 patients studied in detail had reduced diffusing capacities at rest and during exercise. Since the diffusion limitation was not

TABLE 2

CARDIOPULMONARY FUNCTION DATA ON TWELVE PATIENTS WITH DIFFUSE PULMONARY FIBROSIS

Patient	Rest or Exercise	Arterial Blood			D_{CO}*	Pulmonary Arterial Pressure, Mm. Hg.	Cardiac Output L./Min.	Vital Capacity per cent	M.M.F. L./Sec.†	Timed Vital Capacity		V_D/V_T‡ per cent
		Ventilation L. Min.	P_{CO_2} Mm. Hg.	S_{O_2} per cent						1 Second per cent	3 Seconds per cent	
Bur	R	6.6	38	91	12.7	26/8	5.20	66	1.06	64	83	34
	E	22.8	39	87	13.2	32/10	9.12					26
McK	R	4.7	40	87	11.5	13/3	4.00	40	2.16	95	100	23
	E	9.4	37	84	10.8	14/6	5.58					19
Gal	R	6.6	45	88	8.7	29/11	6.45	103	6.90	87.3	100	49
	E	28.8	37	93	8.8	29/15	7.56					50
Gut	R					35/15		74	2.23	79.5	100	
	E	41.5			12.0	44/						
Dau	R	6.8	52	82	3.0	21/?		49	0.29	25.5	61.8	70
Whi	R	6.8	46	90	7.8	25/10	8.83	73	0.61	23	81	42
	E	9.7	47	83	10.9	32/12	8.37					45
Den	R	5.5	50	90	10.5	35/11	5.50	100	1.4	62	87	33
	E	18.7	49	86	13.1	58/20	8.80					33
Nu	R	10.9			9.1	29/?		79	1.76	75	100	
Min	R	9.6	50	86	3.8	26/5	7.52	80	0.93	51	84	61
	E	16.0	51	86	10.0	37/12	10.40					37
Who	R	12.9	45	85	3.6	42/15	13.9	39	1.27	78	96	50
	E	26.0	44	79	12.4	71/23	13.6					47
Gar	R	7.7	55	91	10.0	52/18	4.71	37	1.33	88.2	100	48
	E	14.2	60	84	7.7	95/50	9.00					48
Spe	R	8.9	41	78	3.9	66/?	6.50	79	1.61	73	92	47
	E	25.4	36	66	5.4	85/?	8.55					38

* D_{CO} = diffusing capacity for carbon monoxide in cc. per minute per mm. of mercury
† M.M.F. L/Sec. = maximal midexpiratory flow rate in liters per second
‡ V_D/V_T = lung dead space/tidal volume

necessarily accompanied by measurable alteration of ventilatory function, serious abnormality of arterial blood gas composition, or pulmonary hypertension, impaired diffusion is probably the earliest functional derangment in pulmonary fibrosis. Progression of the fibrosis may then lead to ventilatory impairment, alteration of arterial blood gas composition, or to extensive encroachment on the pulmonary vascular bed with resultant pulmonary hypertension.

The most common ventilatory abnormality in pulmonary fibrosis is reduction of the vital capacity, as seen in 10 of 12 patients in this series. The maximal midexpiratory flow rate was also somewhat reduced in 10 of the 12 patients, suggesting airway obstruction. However, the expiratory delay was corroborated by a reduced three-second vital capacity in only 6 of these 10 patients. In the presence of a reduced vital capacity, a low maximal midexpiratory flow rate is less meaningful than a reduced timed vital capacity as an indication of airway obstruction. Considering both of these values, at least 6 patients had some airway obstruction.

Hyperventilation, usually resulting in a reduced arterial pressure of carbon dioxide (P_{CO_2}) is a common finding in pulmonary fibrosis and is thought to result from reflex effects secondary to the fibrotic process in the lung (1). Six patients (*Dau*, *Nic*, *Min*, *Who*, *Gar*, and *Spe*) revealed increased minute ventilation at rest, and 5 patients (*Bur*, *Gal*, *Gut*, *Who*, and *Spe*) hyperventilated during exercise, as compared with normal subjects studied in this laboratory at rest and during various grades of exercise. In contrast to the hyperventilation and reduced arterial P_{CO_2} usually found in alveolar-capillary block (6), the patients in this series illustrate two other types of physiologic abnormality which may be encountered in pulmonary fibrosis: high arterial P_{CO_2} despite hyperventilation and absence of hyperventilation.

The 5 patients (*Dau*, *Min*, *Who*, *Gar*, and *Spe*)

who had normal or elevated arterial P_{CO_2} despite hyperventilation were found to have an increase of the lung dead space as indicated by a ratio of dead space to tidal volume (Vd/Vt) in excess of 35 per cent. Although the dead space was increased in all 5 patients, different mechanisms may have been responsible. In patients *Dau*, *Min*, and *Spe*, airway obstruction was evident on spirometry. In these patients, alteration of ventilation/perfusion ratios may have increased the dead-space ventilation. Patients *Who* and *Gar*, who also had an increase in lung dead space, had relatively normal ventilatory function so that another mechanism must be sought to explain the increased dead space. It is possible that diffusion was so poor in some alveoli that no gas exchange occurred at all, and thus ventilation to these alveoli represented dead-space ventilation.

Three patients (*McK*, *Whi*, and *Den*) did not hyperventilate. Two of these had carbon dioxide retention and impaired ventilatory function, and the third had neither ventilatory impairment nor carbon dioxide retention. It is conceivable that the increased work of breathing in the 2 patients with ventilatory impairment would have resulted in intolerable dyspnea if these patients had hyperventilated. An analogous situation is thought to exist in patients with emphysema (7).

When the fibrotic process encroaches on the pulmonary vascular bed, pulmonary hypertension may result. Seven patients in this series had pulmonary hypertension at rest, and 6 of 10 on exercise, according to the normal values in use at this laboratory (greater than 27 mm. of mercury systolic at rest, greater than 32 mm. of mercury systolic during exercise). Marked hypertension was present in only 3 cases. Unlike the severe pulmonary hypertension which may complicate pulmonary emphysema and which is primarily due to hypoxia (8), pulmonary hypertension in fibrosis is ascribed to pathologic reduction of the pulmonary vascular bed. As expected, therefore, the hypoxia was less severe in these 3 patients with marked pulmonary hypertension than would be found in emphysematous patients with the same degree of pulmonary hypertension.

The present study indicates that the one consistently abnormal finding in pulmonary fibrosis is reduction of the diffusing capacity. Progression of fibrosis may then lead to ventilatory impairment or pulmonary hypertension. Although severe reduction of vital capacity does not necessarily mean that pulmonary hypertension is present, and although the presence of pulmonary hypertension does not necessarily indicate that marked ventilatory impairment exists, a reduction of the diffusing capacity is to be expected in patients with pulmonary fibrosis when either ventilatory impairment or pulmonary hypertension is detected.

Summary

Measurements of diffusing capacity and vital capacity in 47 patients with pulmonary fibrosis shown roentgenographically have revealed that impaired diffusion is more common than reduction of vital capacity. Detailed studies of 12 patients have revealed consistent reduction of the diffusing capacity despite variable reduction of vital capacity and normal or elevated pulmonary arterial pressure. The most consistent functional abnormality encountered in pulmonary fibrosis is reduction of the diffusing capacity of the lung.

Sumario

La Función Cardiopulmonar en la Fibrosis Pulmonar

Mediciones de la capacidad difusora y de la capacidad vital en 47 enfermos de fibrosis pulmonar visualizada radiográficamente han revelado que el empeoramiento de la difusión es más común que la reducción de la capacidad vital. Estudios pormenorizados de 12 enfermos han revelado reducción constante de la capacidad difusora a pesar de una reducción variable de la capacidad vital y de una presión arteriopulmonar normal o elevada. La anomalía funcional más constante encontrada en la fibrosis pulmonar es la reducción de la capacidad difusora del pulmón.

Resume

La fonction cardio-pulmonaire dans la fibrose pulmonaire

Les mesures de la capacité de diffusion et de la capacité vitale effectuées chez 47 malades atteints de sclérose pulmonaire, mises en évidence par la radiologie, ont révélé que l'altération de la diffusion est plus fréquente que la réduction de la capacité vitale. L'étude détaillée de 12 patients a révélé une réduction constante de la capacité de diffusion malgré une réduction variable de la

capacité vitale et une pression artérielle pulmonaire normale ou élevée. L'anomalie fonctionnelle la plus constante ayant été rencontrée dans la sclérose pulmonaire est la réduction de la capacité de diffusion du poumon.

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THE DIAGNOSIS OF EMPHYSEMA: A PHYSICAL SIGN, A ROENTGENOGRAPHIC SIGN, AND AN OXIMETER TEST[1, 2]

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Introduction

The difficulties encountered in making a diagnosis of diffuse obstructive emphysema have often been stressed. The only essential symptom is dyspnea, which is often found in many other conditions. The diagnosis of emphysema from physical signs is not easy. Fletcher (1) reported the experience of eight physicians who were particularly interested in emphysema and who were asked to examine twenty patients with emphysema. There was considerable disagreement about the presence and absence of physical signs such as barrel-chest, wide subcostal angle, kyphosis, use of accessory muscles of respiration, impaired chest expansion, movement *en bloc*, generalized hyperresonance, impaired cardiac dullness, absent apical impulse, and impaired breath sounds. It was concluded that by careful definition of some of the signs agreement might be increased, but that the clinical diagnosis of emphysema could not be made with any confidence except perhaps in the most advanced cases. The claim that emphysema presented a characteristic roentgenographic appearance has been challenged. Knott and Christie (2) submitted roentgenograms of twenty patients with moderate or advanced emphysema and those of twenty normal subjects to a physician and three radiologists, all of whom had special experience in this field. When given postero-anterior and lateral films in full inspiration and full expiration, all observers correctly diagnosed 70 per cent of the emphysematous and 80 per cent of the control subjects. The films were then re-submitted, but only the full inspiration postero-anterior and lateral views were supplied. Of the group with emphysema, 75 per cent were correctly diagnosed; but only 40 per cent of the normal group were passed as normal. Thus emphysema was incorrectly diagnosed in 12 of 20 normal subjects. It was concluded that a roentgenographic diagnosis of emphysema based on postero-anterior and lateral films on full inspiration was of doubtful significance.

Respiratory function tests are often helpful, but no single test has been specific for the diagnosis of emphysema. In one series (3) the best three tests were intrapulmonary gas mixing, maximal breathing capacity, and the residual volume/total lung capacity ratio. In another series (4), the best results were obtained by a prolonged timed vital capacity, air trapping on the spirogram, and an abnormal single-breath oxygen test. Abnormalities of gas diffusion have been emphasized (5).

The diagnosis of emphysema requires a consideration of many factors, including history, physical examination, and roentgenograms, as well as respiratory function tests. In the present investigation, a physical sign (the position of the inferior right lung border as determined by percussion in the midclavicular line), a roentgenographic sign (the level of the right diaphragm during quiet breathing), and an oximeter test (the 90 per cent desaturation time) have been found valuable in the diagnosis of diffuse obstructive emphysema.

Methods

The inferior right lung border: With the patient in the recumbent or semi-recumbent position, moderately heavy percussion was used down the right midclavicular line, the pleximeter finger being placed parallel to the ribs. A change from resonance to definite dullness indicated the level of the inferior right lung border.

Level of the right diaphragm on the S-roentgenogram: The patient was positioned against the X-ray casette in the usual way, but was given no instructions as to how he should breathe. A chest roentgenogram was then taken. This was termed the "surprise" or "S-roentgenogram." On the roentgenogram a perpendicular line was drawn

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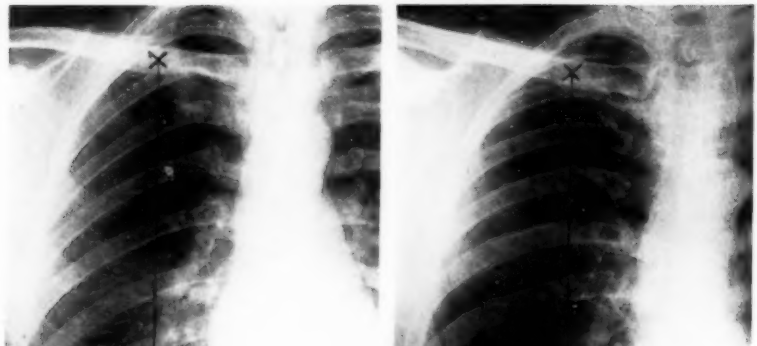

FIG. 1. S-roentgenograms of a normal subject (left) and a patient with emphysema (right), showing method of recording level of right diaphragm. Note low diaphragm of the patient with emphysema.

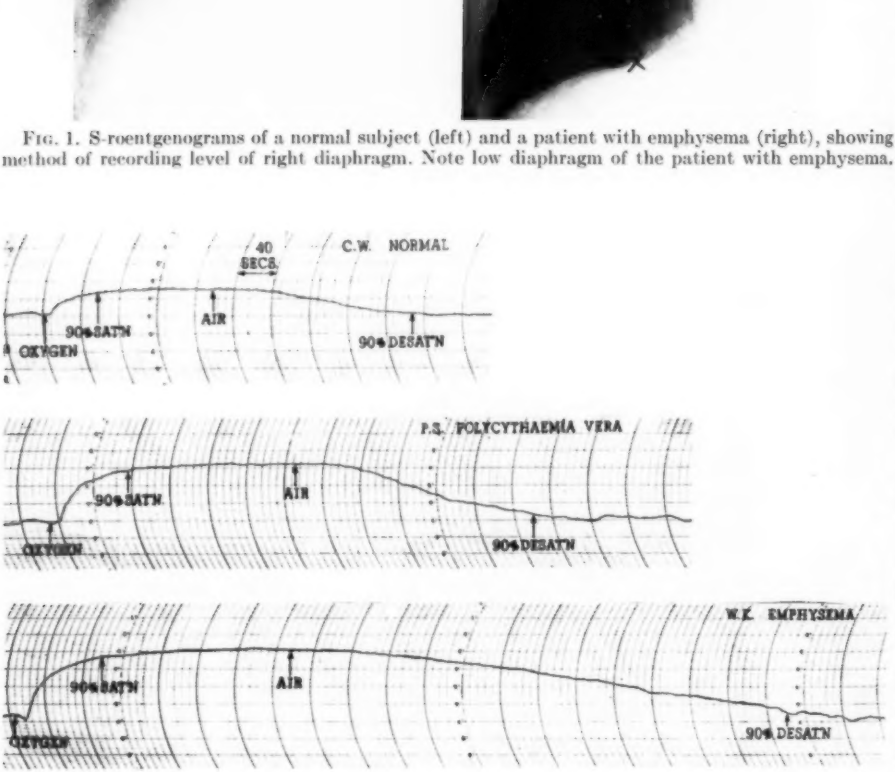

FIG. 2. Oximeter saturation and desaturation curves. Note prolonged fall of arterial oxygen saturation in the patient with emphysema when changed from breathing oxygen to breathing air.

from the point of intersection of the first rib and the clavicle to the dome of the right diaphragm, and this latter point was recorded in relationship to the posterior ribs and interspaces (figure 1). This simple technique did not require the patient's cooperation and should result in comparable roentgenograms. Roentgenograms taken at the end of a quiet inspiration or expiration were not used, as the inspiration or expiration might be very variable in depth in untrained subjects.

The 90 per cent desaturation time: The technique for determining the 90 per cent desaturation time has been described in a previous paper (6). The subject was given oxygen to breathe, and the arterial oxygen saturation was recorded by a direct-writing ear oximeter. After the arterial oxygen saturation had reached a plateau, the subject breathed room air, and gradually the arterial oxygen saturation fell until the original steady state was reached. The 90 per cent desaturation time was the time in seconds from the cessation of oxygen breathing until the arterial oxygen saturation had fallen 90 per cent of the way to the steady state (figure 2). When more than one test was done on an individual subject, the mean of the results was recorded.

In the present investigation, respiratory function tests were performed on many of the normal subjects and on almost all of the patients with pulmonary disease. Standard techniques were used to measure ventilation (6, 7). A closed-circuit helium technique was used to measure the functional residual capacity. A Waters Nitrogen Meter was used for the single-breath oxygen test (8) and for determining the alveolar nitrogen percentage after breathing oxygen for seven minutes (9). The changes in the arterial oxygen saturation on exercise were determined by oximetry (6). Diffusing capacity (D_{CO}) was measured by a single-breath technique (10). The standard normal vital capacity and maximal breathing capacity were calculated from formulas described by Baldwin and associates (9), and the normal residual volume and total lung capacity from formulas described by Needham and his coworkers (11). A standard nomenclature has been used (12, 13).

Material

Two hundred and fifty-eight subjects were divided into four groups.

Group 1: normal subjects. Included in this group were members of the hospital staff and hospital patients who showed no evidence of pulmonary abnormality by history, physical examination, and on roentgenography. Respiratory function tests were done on many of these subjects.

Group 2: patients with definite pulmonary disease in whom examination, roentgenography, and respiratory function tests failed to reveal any evidence of emphysema. A few patients with primarily cardiac disease were included. Represented in this group were patients with sarcoidosis, idiopathic pulmonary fibrosis, silicosis, pulmonary scleroderma, rheumatoid lung, hemosiderosis, tuberculosis, bronchiectasis, pleural thickening, lung cysts, asthma, primary and secondary carcinoma of the lung, pulmonary infarction, pulmonary arteriovenous aneurysm, poliomyelitis, kyphoscoliosis, ankylosing spondylitis, nitrous fume poisoning, chlorine gas poisoning, broken ribs, chest-wall burns, primary pulmonary hypertension, constrictive pericarditis, mitral stenosis, and aortic stenosis.

Group 3: patients in whom the diagnosis of diffuse obstructive emphysema with significant pulmonary fibrosis had been established by clinical and roentgenographic examination, by respiratory function tests and, in some cases, by operation or at autopsy.

Group 4: patients with diffuse obstructive emphysema without significant pulmonary fibrosis as determined by criteria similar to the above.

The diagnosis of the presence or absence of emphysema was made using criteria other than those under study. The presence of emphysema was decided on multiple criteria. Particular attention was paid to a history of cough and dysp-

TABLE 1

Total Number of Subjects in Each Group with Sex, Age, and Number of Subjects Used in Each Test

Group	Total	Males	Females	<40 Years	>40 Years	Right Lung Border	S-Roentgenogram	90 Per Cent Desaturation Time
1	57	29	28	37	20	32	23	55
2	81	57	24	27	54	49	5	79
3	31	29	2	2	29	28	3	31
4	89	81	8	14	75	83	23	87
Total	258	196	62	80	178	192	54	252

Group 1: Normal
Group 2: Pulmonary disease other than emphysema
Group 3: Emphysema with pulmonary fibrosis
Group 4: Emphysema alone

nea, the presence of diminished chest movement, decreased heart sounds and breath sounds in spite of a thin chest wall, decreased diaphragmatic movement and decreased lung darkening on expiration on fluoroscopy, and evidence of an obstructive ventilatory defect with high residual volume and abnormal gas mixing in the absence of asthma. The presence of additional pulmonary fibrosis was suggested by diffuse nonvascular streaky shadows on roentgenography, and in some cases a normal residual volume in the presence of a low total lung capacity and obstruction to air flow.

Included in this study are 65 subjects whose oximeter results have been previously reported (6).

The total number of subjects in each group with sex and age and the number of subjects used in each test are shown in table 1.

Results

The Inferior Right Lung Border in the Midclavicular Line

One hundred and ninety-two subjects were examined. The results are shown in table 2 and illustrated graphically in figure 3. In *Group 1* (normal subjects), the right lung border was at or above the sixth interspace without exception. In *Group 2* (pulmonary disease other than emphysema), 46 patients had right lung borders within the normal range. There were 3 subjects in whom the right lung borders were below the sixth interspace.

A. N., a man of forty-nine years, had a myocardial contusion. Apart from an inferior right lung border at the seventh interspace, the only abnormalities were a slightly raised residual volume and total lung capacity. Large but normal lungs possibly accounted for the low right lung border.

W. B., a 59-year-old male, had a lung cyst occupying most of the right hemithorax, and the right lung border was at the seventh interspace.

W. M., a 28-year-old man, had severe mitral stenosis with hemosiderosis and mild bronchospasm. The right lung border was at the seventh rib.

TABLE 2

Position of Inferior Right Lung Border in Midclavicular Line

(192 Subjects)

Group	Total	Sixth Space and Above	Per Cent Total	Seventh Rib and Below	Per Cent Total
1	32	32	100	0	0
2	49	46	94	3	6
3	28	9	32	19	68
4	83	9	11	74	89

Group 1: Normal
Group 2: Pulmonary disease other than emphysema
Group 3: Emphysema with pulmonary fibrosis
Group 4: Emphysema alone

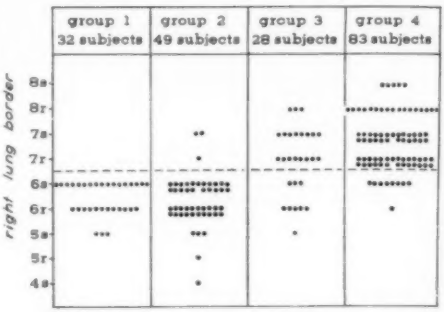

Fig. 3. The position of the inferior right lung border, determined by percussion, in the midclavicular line. Broken line attempts to separate subjects with emphysema (above the line) from those without emphysema (below the line).
Group 1: Normal
Group 2: Pulmonary disease other than emphysema
Group 3: Emphysema and pulmonary fibrosis
Group 4: Emphysema alone
r = rib; s = interspace.

In *Group 3* (emphysema and pulmonary fibrosis), there were 9 cases with the right lung borders at or above the sixth interspace, and 19 cases with right lung borders below the sixth interspace. No obvious distinction could be made clinically or by pulmonary function tests between those with normal and those with low inferior right lung borders.

In *Group 4* (emphysema), there were 9 patients with right lung borders at or above the sixth interspace and 74 patients with right lung borders below the sixth interspace. Of the exceptional 9 patients, 6 were classed as having slight emphysema, one as having moderate emphysema, one had a right diaphragmatic paralysis, and one had unilateral emphysema of the left lung only. This latter patient might have been placed more accurately in *Group 2*.

In summary, normal subjects and patients without emphysema usually had inferior right lung borders at or above the sixth interspace,

whereas patients with emphysema usually had right lung borders below the sixth interspace. Exceptions to this rule were frequent in patients with emphysema and significant pulmonary fibrosis.

Level of the Right Diaphragm on the S-Roentgenogram

Roentgenograms were obtained on 54 subjects, and the results are shown in table 3 and figure 4. Normal subjects and patients without emphysema usually show the level of the right diaphragm at or above the tenth posterior interspace

TABLE 3
LEVEL OF RIGHT DIAPHRAGM ON S-ROENTGENOGRAM
(54 Subjects)

Group	Total	Tenth Space and Above	Eleventh Rib and Below
1	23	22	1
2	5	5	0
3	3	1	2
4	23	13	10

Group 1: Normal
Group 2: Pulmonary disease other than emphysema
Group 3: Emphysema with pulmonary fibrosis
Group 4: Emphysema alone

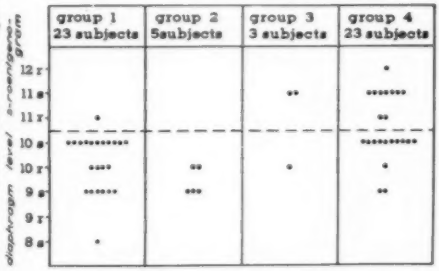

FIG. 4. The position of the right diaphragm (related to posterior ribs and interspaces) on the S-roentgenogram (quiet breathing). Broken line attempts to separate subjects with emphysema (above the line) from those without emphysema (below the line).
Group 1: Normal
Group 2: Pulmonary disease other than emphysema
Group 3: Emphysema and pulmonary fibrosis
Group 4: Emphysema alone
r = rib; s = interspace.

Thirteen of the 23 patients with emphysema showed the level of the right diaphragm to be within the normal range, and 6 of these had advanced emphysema. Ten of the patients with emphysema had the right diaphragm at or below the eleventh rib.

It was concluded that, if the right diaphragm was at the eleventh rib or below on the surprise roentgenogram, then it was very likely that the patient had emphysema. However, if the right diaphragm was above this level, emphysema could not be excluded.

The 90 Per Cent Desaturation Time

The 90 per cent desaturation time was measured on 252 subjects, and the results are shown in table 4 and figure 5. In *Group 1* there were 54 subjects with 90 per cent desaturation times less than 350 seconds, and only one with a value greater than 350 seconds. This exception was a 67-year-old male with carcinoma of the tongue, whose chest was normal on physical examination and whose pulmonary function tests were normal except for a 90 per cent desaturation time of 472 seconds. He was the only normal subject to have a low right diaphragm on the S-roentgenogram (eleventh rib).

In *Group 2*, there were 74 patients with 90 per cent desaturation times of less than 350 seconds, and 5 patients with 90 per cent desaturation times greater than 350 seconds. Four of these exceptions had good pulmonary function. The diagnoses were aortic stenosis, silicosis and auricular fibrillation, primary pulmonary hypertension, and healed broken ribs. One had a moderate restrictive ventilatory defect due to severe bilateral bronchiectasis.

In *Group 3*, 30 patients had 90 per cent desaturation times of more than 350 seconds, and only one less than 350 seconds. The exception was a 48-year-old miner with moderately advanced silicosis and bronchogenic carcinoma of the right upper lobe. Pulmonary function tests showed a restrictive ventilatory defect without evidence of emphysema. Histologic sections from the right lung after pneumonectomy and of the left lung (which was removed at autopsy after death due to secondary deposits) showed very early emphysema associated with pulmonary fibrosis.

In *Group 4*, 84 patients had 90 per cent desaturation times greater than 350 seconds, and

TABLE 4
90 PER CENT DESATURATION TIME IN SECONDS
(252 Subjects)

Group	Total	350 and Less	Per Cent Total	351 and More	Per Cent Total	Mean ± S.D.*
1	55	54	98	1	2	229 ± 60
2	79	74	94	5	6	252 ± 62
3	31	1	3	30	97	534 ± 166
4	87	3	3	84	97	596 ± 192

* S.D. = Standard deviation
Group 1: Normal
Group 2: Pulmonary disease other than emphysema
Group 3: Emphysema with pulmonary fibrosis
Group 4: Emphysema alone

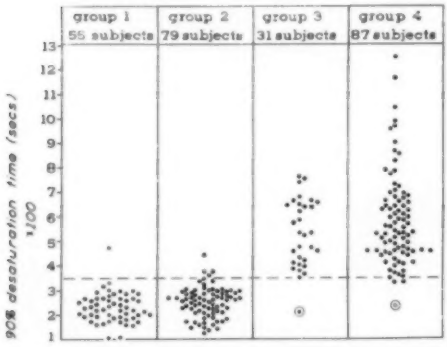

FIG. 5. The 90 per cent desaturation time. The broken line is at 350 seconds and separates subjects with emphysema (above the line) from those without emphysema (below the line).
Group 1: Normal
Group 2: Pulmonary disease other than emphysema
Group 3: Emphysema and pulmonary fibrosis
Group 4: Emphysema alone
◉ Patient (T.K.) with early emphysema shown only by histology of resected lung.
◎ Patient with left-sided emphysema.

3 had values less than 350 seconds. One of these had unilateral left-sided emphysema and is the patient mentioned as having a normal right lung border. The other 2 patients were considered to have early emphysema, one of whom had a normal, and the other a low, right lung border; their results were 332 seconds and 336 seconds, that is, just below the emphysema level.

It may be seen in table 4 that the mean and the standard deviations are very similar for *Groups 1* and *2*, and the difference between these groups is not statistically significant (P between 0.1 and 0.05). There is a significant difference when *Groups 3* and *4* are compared with *Group 1* ($P < 0.001$).

In summary, if a patient has a 90 per cent desaturation time over 350 seconds, it is very likely that emphysema is present. If the desaturation time is less than 350 seconds, emphysema is usually not present.

DISCUSSION

The level of the inferior right lung border in the midclavicular line has been shown to be of value in determining the presence or absence of emphysema. It seemed important to decide if this physical sign could be easily and accurately elicited by doctors who did not have a special interest or training in diseases of the chest. To determine this, the right lung border was recorded on 10 patients; then four senior interns were asked to percuss out this level on the same patients. The interns were told to use moderately heavy percussion in the right midclavicular line and to record a definite change in percussion note. None of the interns had previously paid attention to this physical sign. No history or other examination was permitted, nor any comparison of results allowed. The results are shown in table 5. There was, on the whole, good agreement and the sign appeared to be easy to elicit accurately. In 6 of the 10 patients, all five observers were in agreement. In 3 patients, one intern disagreed with the other three interns and the writer. In one patient, two interns disagreed with the other two interns and the writer. With a little more experience, greater agreement might have occurred.

It was thought that it might be of interest to try and relate the position of the right lung border to the residual volume. This was done in 90 subjects (24 in *Group 1*; 19 in *Group 2*; 15 in *Group 3*; 32 in *Group 4*). There were only 6 subjects with high residual volumes and normal right lung borders, and in 5 of these the residual volume was between 120 and 135 per cent of normal. Only 6 subjects had low right lung borders with residual volumes below 120 per cent of normal and, of these, 5 had emphysema with pulmonary fibrosis. With these exceptions, the rule was that patients with right lung borders at or above the sixth interspace had normal or

TABLE 5
THE RIGHT LUNG BORDER DETERMINED INDEPENDENTLY BY FOUR SENIOR INTERNS AND THE WRITER

Patient	Sixth Space and Above	Seventh Rib and Below
1	3 •	1
2	3 •	1
3	2 •	2
4	4 •	0
5	4 •	0
6	3 •	1
7	4 •	0
8	0	4 •
9	0	4 •
10	0	4 •

The numerals represent the number of interns in agreement.
The dots indicate the position of the right lung border as determined by the writer.

low residual volumes, and those with right lung borders below the sixth interspace had high residual volumes.

The change from resonance to dullness might occur at an abnormally low level in the absence of emphysema: if there was a large cyst in the right lung, if there was a right pneumothorax, a pneumoperitoneum, hepatodiaphragmatic interposition of the colon, or severe ileus. The inferior right lung border would probably be low in the presence of acute asthma without emphysema. A normal right lung border in the presence of emphysema might occur if there was a paralyzed right diaphragm, if the emphysema was unilateral on the left side, if the emphysema was very slight, or if there was marked pulmonary fibrosis in addition to the emphysema.

The level of the right diaphragm on a surprise or resting roentgenogram was of limited value in the diagnosis of emphysema. In the usual full inspiration chest roentgenogram, the normal subject might have quite a low diaphragm and it was hoped that in the pulmonary resting position, during quiet breathing, the low diaphragms of patients with emphysema might be easily distinguished from the diaphragms of subjects without emphysema. This was unfortunately not the case, as shown in figure 4. The level of the diaphragm was related to the residual volume in 44 subjects (21 in *Group 1*; 4 in *Group 2*; 2 in *Group 3*; 17 in *Group 4*). Seven subjects had diaphragms at or below the eleventh rib and, of these, one had a low residual volume and the other 6 had high residual volumes. Most of the patients with high residual volumes had diaphragms at the normal level. A low diaphragm at or below the eleventh rib on this roentgenogram was usually associated with a high residual volume but, if the level was above the eleventh rib, then the residual volume might be high, normal, or low.

The value of the 90 per cent desaturation time in the diagnosis of emphysema has been confirmed in the present investigation. This measurement was of particular value when emphysema and pulmonary fibrosis were present. The factors which might be the cause of the prolonged 90 per cent desaturation time in patients with emphysema have been discussed in a previous paper (6). The relationships were investigated of the 90 per cent desaturation time to the forced vital capacity, forced expiratory volume (one second), maximal breathing capacity, residual volume, single-breath oxygen mixing test, seven-minute nitrogen washout, and fall of the arterial oxygen saturation on exercise (table 6).

There was little relationship between the 90 per cent desaturation time and the forced vital capacity. Many subjects with normal vital capacities had prolonged 90 per cent desaturation times, and many subjects with low vital capacities had normal 90 per cent desaturation times.

The 90 per cent desaturation time did bear a relationship to the forced expiratory volume (one-second), expressed as a percentage of the total forced vital capacity: the lower the one-second forced expiratory volume, the higher the 90 per cent desaturation time. This was confirmed statistically ($r = -0.76$; $P < 0.001$).

There was little relationship between the 90 per cent desaturation time and the maximal breathing capacity. Although only a few patients with normal maximal breathing capacities had high 90 per cent desaturation times, there were many with low maximal breathing capacities and normal 90 per cent desaturation times.

There was a correlation between the 90 per cent desaturation time and residual volume. Most of the patients with high residual volumes also had high 90 per cent desaturation times. Only 4 patients with high residual volumes had low 90 per cent desaturation times and 3 of these had residual volumes between 120 and 140 per cent.

TABLE 6

NUMBER OF SUBJECTS IN EACH GROUP IN WHICH THE 90 PER CENT DESATURATION TIME WAS COMPARED WITH TESTS OF PULMONARY FUNCTION

Group	F.V.C.	$F.E.V._{1.0}$	M.B.C.	R.V.	Single-breath Oxygen Mixing	7-Minute Nitrogen Washout	Fall of A.O.S. on Exercise
1	40	29	38	23	25	25	40
2	78	43	76	19	18	19	71
3	31	18	31	15	7	12	29
4	87	62	85	32	23	29	76
Total	236	152	230	89	73	85	216

F.V.C. = Forced vital capacity
$F.E.V._{1.0}$ = Forced expiratory volume (1-second)
M.B.C. = Maximal breathing capacity
R.V. = Residual volume
A.O.S. = Arterial oxygen saturation
Group 1: Normal
Group 2: Pulmonary disease other than emphysema
Group 3: Emphysema with pulmonary fibrosis
Group 4: Emphysema alone

Most of the patients with low residual volumes and high 90 per cent desaturation times had emphysema and pulmonary fibrosis. In the patients with emphysema alone, the higher the residual volume the more prolonged the 90 per cent desaturation time ($r = +0.58$; $P < 0.001$).

Most of the patients with abnormal gas mixing, as shown by the single-breath oxygen test, also had prolonged 90 per cent desaturation times. Only 4 subjects had normal gas mixing and prolonged 90 per cent desaturation times. In the emphysema group, with or without fibrosis, there was a statistical correlation between the degree of abnormality of gas mixing and the degree of prolongation of the 90 per cent desaturation time: the greater the abnormality of mixing, the more prolonged the 90 per cent desaturation time ($r = +0.59$; $P < 0.001$).

There was a relationship between abnormal gas mixing (determined by the seven-minute nitrogen washout test) and prolongation of the 90 per cent desaturation time. There were 2 subjects who had slightly abnormal mixing test reactions in which the 90 per cent desaturation times were normal. All other patients in whom this mixing test was abnormal also had abnormally prolonged 90 per cent desaturation times. This mixing test is less sensitive than the single-breath oxygen test and probably is abnormal only in those patients with the more severe mixing defects.

There was no relationship between the fall of arterial oxygen saturation on exercise and the 90 per cent desaturation time. Four subjects in *Group 2* had little abnormality of ventilation, low diffusion capacities (D_{CO}), and normal 90 per cent desaturation times. Seven other subjects probably had alveolar capillary block, as demonstrated by an abnormal fall of the arterial oxygen saturation on exercise in the presence of normal or only slightly reduced ventilation, and all of these had normal 90 per cent desaturation times.

These results showed that the prolongation of the 90 per cent desaturation time was not related to decreased vital capacity and maximal breathing capacity as such, nor to alveolar capillary block. The suggestion made previously (6) that a large residual volume and inefficient gas mixing were the most important factors in the prolongation of the 90 per cent desaturation time was confirmed. Because of the large functional residual capacity, the patient with emphysema commenced the 90 per cent desaturation time test with a larger volume of oxygen in his lungs than did the subject without emphysema. In addition, there was a less efficient washout mechanism because of poor gas mixing and obstruction to air flow. Oxygen was probably slowly absorbed from poorly ventilated areas where it remained in high concentration and, possibly, oxygen from these areas enriched the gas mixture to other portions of the lung during the washout period.

Patients with all grades of emphysema showed prolonged 90 per cent desaturation times, and

the test was abnormal in the presence or absence of pulmonary fibrosis. The presence of even early emphysema resulted in prolongation of the 90 per cent desaturation time. This diagnosis was made in 14 of the 84 patients in *Group 4*. The 90 per cent desaturation time was abnormal in 12 of these cases of early emphysema.

In the diagnosis of emphysema, the right lung border and the 90 per cent desaturation tests were of considerable value. For example, a 56-year-old male had a "coin" lesion in the upper lobe of the right lung. He complained of some dyspnea on exertion. On examination, rhonchi were present and the right lung border was at the seventh rib. Pulmonary function tests: one-second forced expiratory volume, 80 per cent of total; forced vital capacity, 111 per cent; maximal breathing capacity, 115 per cent; no air trapping on the spirogram; seven-minute nitrogen washout, 3 per cent; fall of arterial oxygen saturation on exercise, 2.4 per cent; 90 per cent desaturation time, 729 seconds. Histologic sections of the lung removed at operation showed moderate diffuse emphysema.

The position of the inferior right lung border and the 90 per cent desaturation time has been correlated in 187 subjects (30 in *Group 1*, 47 in *Group 2*, 28 in *Group 3*, 82 in *Group 4*). All of the patients who had low right lung borders and prolonged 90 per cent desaturation times had emphysema. Only one patient with emphysema had a low right lung border and a normal 90 per cent desaturation time. In 15 patients with emphysema (8 of whom had additional pulmonary fibrosis), the right lung borders were normal but the 90 per cent desaturation times were prolonged. Both the right lung borders and the 90 per cent desaturation times were within normal limits in only 2 patients with emphysema. One of these was the patient with unilateral emphysema, and the other had slight emphysema. The right lung border and 90 per cent desaturation time were normal in one patient with emphysema and pulmonary fibrosis. This was the patient with silicosis and carcinoma in whom early emphysema was diagnosed on histology only. Three patients with pulmonary disease other than emphysema had low right lung borders, but in all of these the 90 per cent desaturation times were normal. One normal subject and 4 patients with pulmonary disease other than emphysema had prolonged 90 per cent desaturation times, but the right lung borders were normal.

In conclusion, if a patient had an inferior right lung border in the midclavicular line below the sixth interspace, and if the 90 per cent desaturation time was greater than 350 seconds, then he almost certainly had emphysema. If, in addition, the level of the right diaphragm on the special resting roentgenogram was at or below the eleventh rib, then this was confirmatory evidence in favor of emphysema.

Summary

The inferior right lung border was determined by percussion in the midclavicular line in 192 subjects, and was shown to be at or above the sixth interspace in normal subjects and in patients without emphysema. Patients with emphysema usually had right lung borders below the sixth interspace but, if significant pulmonary fibrosis was present, then the right lung border was often within the normal range.

"Surprise" roentgenograms were taken of 54 subjects who were breathing normally. If the level of the right diaphragm on this S-roentgenogram was at or below the eleventh rib, then emphysema was probably present. If the diaphragm was above this level, emphysema could not be excluded.

The 90 per cent desaturation time was recorded by an ear oximeter on 252 subjects. Subjects without emphysema showed 90 per cent desaturation times of less than 350 seconds, irrespective of the presence or absence of other pulmonary disease. Nearly all of the subjects with emphysema, whether pulmonary fibrosis was present or not, showed 90 per cent desaturation times of more than 350 seconds.

These tests were related to other tests of pulmonary function. The prolongation of the 90 per cent desaturation time was shown to be related to a high residual volume, obstruction to air flow, and abnormalities of intrapulmonary gas mixing.

Sumario

El Diagnóstico del Enfisema: Un Signo Físico, un Signo Radiográfico y una Prueba Oximétrica

Se determinó el borde inferior del pulmón derecho por la percusión en la línea mesoclavicular en 192 sujetos, demostrándose que quedaba en el sexto espacio intercostal o más arriba en los sujetos normales y en los enfermos sin enfisema.

Los enfisematosos suelen tener un borde del pulmón derecho más abajo del sexto espacio, pero cuando existía fibrosis pulmonar de importancia, el borde del pulmón derecho quedaba entonces a menudo dentro de los límites normales.

Se tomaron radiografías "inesperadas" de 54 sujetos que respiraban normalmente. Si el nivel de la porción derecha del diafragma en esa radiografía-I queda en la undécima costilla o más abajo, había probablemente enfisema. Si el diafragma quedaba más arriba de dicho nivel, no puede excluirse en enfisema.

Se registró el tiempo de desaturación de 90 por ciento con un audio-oxímetro en 252 sujetos. Los sujetos sin enfisema registraron un tiempo de desaturación de 90 por ciento de menos de 350 segundos, independientemente de si había o no otra afección pulmonar. Casi todos los enfisematosos, ya hubiera o no fibrosis pulmonar, revelaron un tiempo de desaturación de 90 por ciento superior a 350 segundos.

Estas pruebas se relacionaron con otras pruebas de la función pulmonar. Se demostró que la prolongación del tiempo de desaturación de 90 por ciento se correlacionaba con un alto volumen residual, oclusión de la corriente de aire y anomalías en la mezcla intrapulmonar de gases.

Resume

Le diagnostic de l'emphysème—Un signe physique, un signe radiographique, et un test oxymétrique

La limite inférieure du poumon droit a été déterminée chez 192 personnes par la percussion suivant une ligne partant du milieu de la clavicule; il a été constaté qu'elle se trouvait au niveau du sixième espace intercostal ou au-dessus chez les malades ne présentant pas d'emphysème. Chez les malades atteints d'emphysème, la limite inférieure du poumon droit se trouvait en général au-dessous du sixième espace intercostal; cependant dans les cas s'accompagnant de sclérose pulmonaire marquée, le bord inférieur du poumon droit ne dépassait souvent pas la limite normale.

Des radiographies furent prises "à l'improviste" chez 54 personnes qui respiraient normalement. Si le niveau du diaphragme droit sur cette radiographie se trouvait au niveau de la onzième côte ou au-dessous, il était probable qu'il existait de l'emphysème. Si le diaphragme se trouvait au-dessus de ce niveau, la possibilité d'emphysème n'était pas exclue.

Le temps de désaturation à 90% a été enregistré avec un oxymètre à oreille chez 252 personnes. Ce temps était de moins de 350 secondes chez les personnes ne présentant pas d'emphysème, qu'elles soient atteintes ou non d'une autre affection pulmonaire. Le temps de désaturation à 90% était de plus de 350 secondes chez presque tous les sujets ayant de l'emphysème, qu'ils présentent ou non de la sclérose pulmonaire.

Ces tests furent comparés à d'autres tests du fonctionnement pulmonaire. Il a été constaté que la prolongation du temps de désaturation à 90% était en rapport avec un volume résiduel élevé, l'obstruction du débit de l'air et des anomalies du mélange gazeux interpulmonaire.

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CHEMOPROPHYLAXIS IN CHRONIC OBSTRUCTIVE PULMONARY EMPHYSEMA[1]

A Twelve-Week Study with Erythromycin

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Introduction

Infections in the lower respiratory passages are often considered important in the pathogenesis and progress of chronic obstructive pulmonary emphysema (1, 2). Inhalation of irritants from industrial air pollution, tobacco smoke, and functional abnormalities have been similarly implicated (3–5). The relative importance of these factors remains to be determined. Evaluation of the ultimate benefit of any form of treatment is hampered by our incomplete knowledge of the etiology and natural history of this disease. Therefore, most treatment at present is directed toward the temporary alleviation of manifestations of the far advanced condition.

Chronic bronchitis is a disease whose characteristics merge in many respects with those of chronic obstructive emphysema. In certain large cities of Great Britain, where bronchitis is a major health problem, *Hemophilus influenzae* and other pathogenic organisms have been found in the purulent sputum often associated with this condition (6, 7). Suppression of *H. influenzae* and pneumococci in the sputum with appropriate chemotherapy has been believed to be associated with symptomatic improvement. The prophylactic use of an antimicrobial drug for long periods, more than two years in some cases, is purported to reduce the number, severity, and duration of intercurrent respiratory infections (7–10). Data regarding pulmonary functions do not form a conspicuous part of any of these studies, most of which have depended upon double-blind evaluation of subjective responses to antimicrobial drugs.

The purpose of the present study was to administer erythromycin for three months to a group of patients with chronic obstructive emphysema and to observe the effect on pulmonary function, sputum, and symptoms.

Erythromycin was chosen because its antibacterial spectrum encompasses *Hemophilus influenzae*, *Diplococcus pneumoniae*, and other common respiratory pathogens; the hazard of drug-resistant superinfection is low; and side effects are minimal (11, 12).

Methods and Materials

Subjects: Twenty patients (17 males and 3 females) with chronic diffuse obstructive pulmonary disease were selected at random over a three-week period at the Chest and Allergy Clinics of the King County Hospital. The only criterion for selection was that the patient had been demonstrated to have diffuse pulmonary obstruction irreversible by any previous means of therapy. All of these patients had sought aid for moderate or severe dyspnea of one to fifty years' duration (mean: seventeen years). Their age range was from thirty-nine to seventy-eight years (mean: sixty years).

Medications and schedule of examinations: Two pretreatment evaluations were conducted with a one-week interval between them. Subsequent examinations were performed after one, two, four, eight, and twelve weeks of treatment (March to June, 1958). After the first pretreatment evaluation, the patients were matched in pairs on the basis of similar clinical characteristics.[5] Erythromycin tablets, 250 mg., were assigned to one and a placebo of identical appearance to the other member of each pair.[6] The tablets were to be taken four times daily. Neither the patients nor the physicians conducting the examinations were informed of how the patients were matched or distributed into treatment and control groups. The patients continued to receive bronchodilators, expectorants, and other medications as needed.

Sputum: At each visit sputum was collected in the presence of the examiner who noted the gross character of the sample. The sputum was im-

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[4] Daland Fellow, American Philosophical Society.

[5] This matching was done by Dr. Norman Arcese, whose aid is gratefully acknowledged.

[6] Erythromycin as Ilotycin and placebo tablets were generously supplied by Eli Lilly & Co.

mediately examined by Gram stain and cultured on blood agar at 37°C.

Subjective evaluation: Patients were asked to compare their general state of health, exercise tolerance, cough, and sputum volume and character relative to their pretreatment status. Numerical scores for these statements were obtained by recording 0 for no change, + or − 1, 2, 3 for minimal, moderate, or marked change, respectively, towards better or worse. Notation was made of side effects, appearance of new acute respiratory infections, and dosage schedules of all drugs taken.

Objective evaluation: A 9-liter spirometer[7] with the valves and carbon-dioxide–absorbent canister removed was used to test vital capacity (13), one-second timed vital capacity (14), and maximal breathing capacity (15). The tests were done at least three times and the best performances were recorded. Walking ventilation was determined by collecting expired air in a rubber bag during three minutes of walking on a level treadmill at 2 miles per hour. The relation of this ventilation to maximal breathing capacity constitutes the walking ventilation index (16). Maximal expiratory flow was recorded with a Puffmeter (17). The average of the best three of five trials was recorded. Gas volumes were corrected for body temperature, atmospheric pressure, and water vapor saturation.

Results

Sputum Character and Bacteriology

The changes in the appearance of sputum are recorded in table 1. It may be seen that the patient's evaluation of the sputum character was not always confirmed by observation of the specimen produced during the examination. Sputum volumes ranged from 5 to 400 ml. per day. An increase in sputum volume took place in 2 of the erythromycin-treated group and in 4 of the placebo group.

A total of 113 sputum samples was collected. Thirty-eight of these were either purulent or mucopurulent. The predominant bacterial flora of these 38 samples is shown in table 2. There seemed to be an association between the presence of purulent or mucopurulent sputum and predominance of pneumococci. The frequency of isolation of various microorganisms from the sputum cultures is indicated in table 3. It should be noted that *N. catarrhalis* was listed only when it was the predominant organism on the plate; this occurred in almost 50 per cent of the samples from the erythromycin-treated group. The other organisms were listed on the basis of isolation, whether or not they were predominant. *H. influenzae* was not isolated from any of the cultures. Erythromycin eliminated pneumococci; and no increase in Gram-negative bacilli followed the administration of erythromycin. There was a significant growth of fungus (Monilia) in only one culture, from a patient who received the placebo. The persistence of beta hemolytic streptococci in the sputum of erythromycin-treated patients was of interest. Although seldom predominant, this organism grew from at least 50 per cent of the cultures obtained immediately preceding intercurrent respiratory infections. Since no immunologic identification or drug-susceptibility studies were done, it is possible that these bacteria were hemolytic enterococci.

TABLE 1

Changes in Sputum Character during Study Period

Sputum Character	Treatment			
	Erythromycin (10 Patients)		Placebo (10 Patients)	
	Patient's Statement	Examiner's Observation	Patient's Statement	Examiner's Observation
Originally purulent	6	4	5	3
Remained purulent	2	2	3	1
Cleared	4	2	2	2
Originally clear	4	6	5	7
Remained clear	3	4	4	3
Became purulent	1	2	1	4

Subjective Evaluation

Side effects: Almost all of the patients had some complaint which was attributed to the medication taken, whether it was erythromycin or placebo. These ranged from euphoria to depression, from urinary retention to polyuria; and included hemoptysis, sweating, belching, weakness, and backache. The only differences found in these complaints was a laxative action noted by 3 of the subjects who took erythromycin. This effect was transient in one, persistent in 2, and annoying to none.

Intercurrent respiratory illness: Intercurrent res-

[7] Manufactured by W. E. Collins Co., Boston, Massachusetts.

TABLE 2

PREDOMINANT ORGANISM IN PURULENT OR MUCOPURULENT SPUTUM IN RELATION TO TREATMENT

Cultures of Purulent or Mucopurulent Sputum from:	Total Number of Purulent and Mucopurulent Samples	Predominant Organism (Number of Cultures)				
		Pneumococcus	Beta hemolytic Streptococci	*Neisseria Catarrhalis*	"Usual Flora"	Others
Erythromycin group						
Control period (total cultures, 18)	9	3	0	0	4	2
Treatment period (total cultures, 36)	8	0	1	4	3	0
Placebo group						
Control period (total cultures, 19)	8	2	2	0	2	2
Treatment period (total cultures, 40)	13	4	3	1	3	2

TABLE 3

FREQUENCY OF BACTERIAL ISOLATIONS IN RELATIONSHIP TO TREATMENT
(All Sputum Samples)

	Total Number of Cultures	Pneumococcus	Beta Hemolytic Streptococci	Predominately *N. Catarrhalis*	Gram-negative Rods	Non-hemolytic staphylococci
Erythromycin						
Control	18	5 (28%)	4 (22%)	1 (6%)	3 (17%)	0
Treatment	36	1 (3%)	10 (28%)	17 (47%)	4 (11%)	3 (8%)
Placebo						
Control	19	5 (26%)	9 (47%)	0	6 (32%)	0
Treatment	40	8 (20%)	3 (32%)	3 (8%)	8 (20%)	9 (23%)

piratory illness was judged to be present when the patient complained of a "cold," "flu," or marked increase in rhinorrhea, "sinusitis," cough, sputum, or dyspnea, with or without fever. The incidence of acute respiratory illness during the treatment period was the same in both groups. Among those treated with erythromycin, 7 patients had 8 illnesses with an average duration of thirteen days. Eight of the placebo-treated group suffered eight illnesses lasting an average of fifteen days. Two patients in each group required hospitalization for these, during which time the medication was discontinued. Both of the erythromycin-treated patients who were hospitalized had markedly purulent sputum with a pure culture of *Neisseria catarrhalis*.

General state of health: In figure 1 is illustrated graphically the numerical grading of the patient's response to the question, "In general, how do you feel now in comparison to the time before you were taking the medicine?" It may be seen that there were no significant differences between early and late effects of treatment. Similar results were obtained for exercise tolerance, cough, and sputum, as may be seen in figure 2, which shows a comparison of symptoms before treatment with those near the end of three months of treatment. It is quite clear that there was no difference between the two groups. A preponderance of patients noting improvement is present in both groups. A comparison of the members of each matched pair is also shown in figure 2.

Objective Evaluation

Examination: Physical examinations, including appraisal of cough and auscultation of the chest on each visit, showed no significant differences between the treated and the control groups. Auscultatory findings correlated poorly with the patients' subjective complaints and pulmonary function tests.

Pulmonary function tests: There was a wide

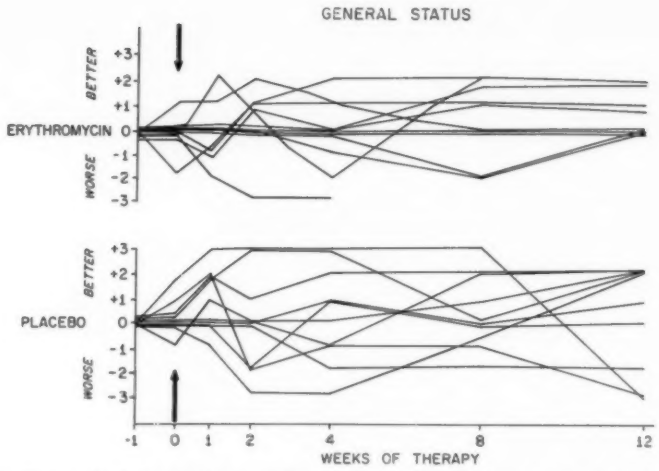

FIG. 1. Composite graph of changes in general state of health for each patient during the study period.

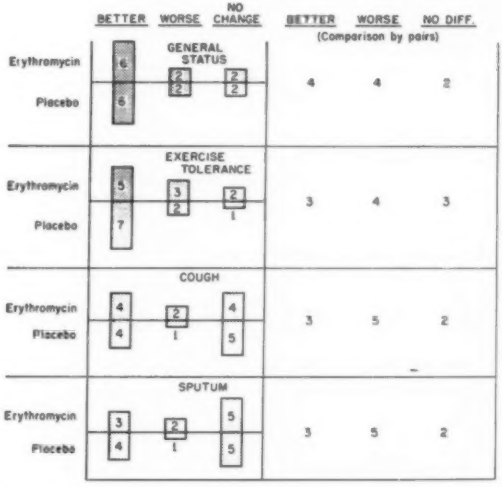

FIG. 2. Comparison of symptoms before treatment with those near the end of treatment.

range in the values of the gross ventilatory functions initially, as would be anticipated from the spread of severity and duration of symptoms in the subjects. The maximal breathing capacity in various patients ranged from 15 to 75 liters per minute (mean: 35). The maximal breathing capacity values for each patient throughout the study are shown in figure 3; plots of maximal expiratory flow rate, one-second timed vital capacity, and vital capacity were similar. Measurements of walking ventilation index showed marked variation from time to time. Several of the patients were unable or unwilling to walk on the treadmill after the first attempt; others were unable to complete the prescribed walking period. A comparison of pulmonary function changes in the two groups during the course of the study is illustrated in figure 4, in which the individual

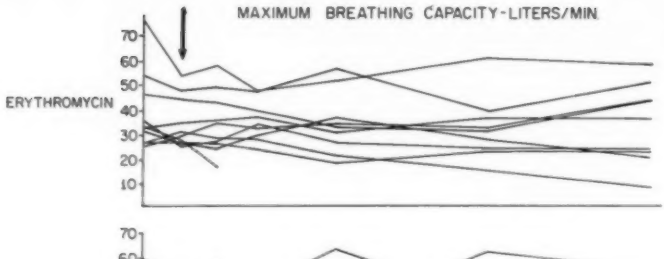

FIG. 3. Composite graph of maximal breathing capacity for each patient during the study period.

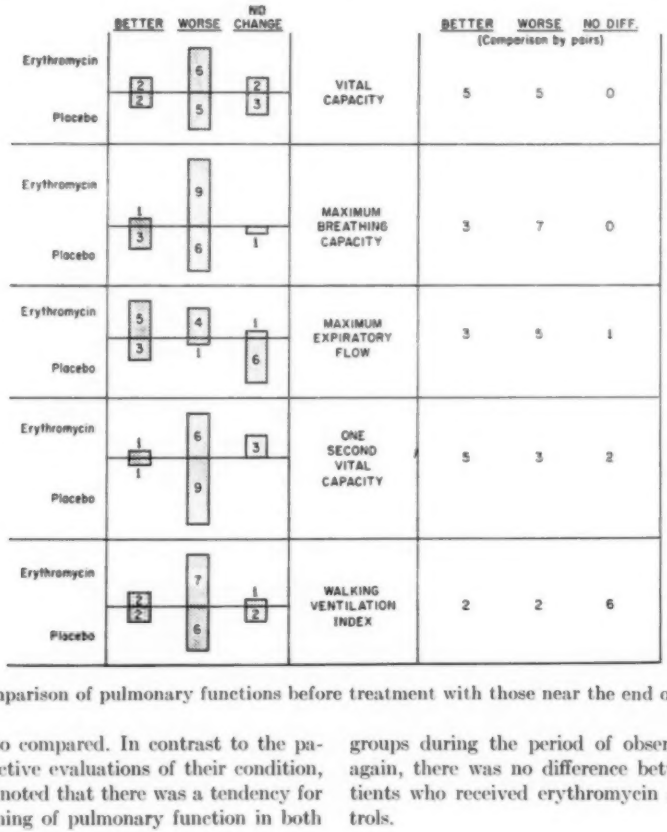

FIG. 4. Comparison of pulmonary functions before treatment with those near the end of treatment.

pairs are also compared. In contrast to the patients' subjective evaluations of their condition, it should be noted that there was a tendency for slight worsening of pulmonary function in both groups during the period of observation. Here again, there was no difference between the patients who received erythromycin and the controls.

Discussion

The conclusions reached from a study such as this must necessarily be tempered by the small number of subjects in each group and the relatively short duration of the experiment. It is possible that observations made during the winter rather than during the spring would have produced different results. Nevertheless, it is quite evident that both the drug-treated and control groups ran identical courses. Only in the effect on bacterial flora of the sputum was there a significant difference; and, in contrast to the experiences described in chronic bronchitis, this seemed to have little correlation with subjective or objective changes in the disease.

There is much confusion about the interpretation of sputum examinations and cultures in chronic pulmonary disease. Mulder has stressed the need for washing sputum samples to eliminate oral contaminants (18), while Brown and associates have concluded that significant contamination of secretions of the lower respiratory tree did not occur on passage through the nasopharynx (19). After a thorough review of existing data, Knight and White suggest that the microorganisms in sputum may be derived from the usual flora of the nasopharynx, and any study of pathogenic agents responsible for bronchitis should take cognizance of this possibility (20). More recently, Pecora and Yegian have shown the bronchi and lungs to be frequently sterile in chronic diseases of the lower respiratory tract (21). These conflicting conclusions do not allow reliable interpretation of sputum studies.

The failure of antimicrobial therapy to produce any demonstrable effect in this group of patients with chronic obstructive pulmonary emphysema emphasizes the fact that, although the two conditions merge in certain characteristics, chronic bronchitis and chronic obstructive emphysema should not be considered identical. Chronic bronchitis in Great Britain is predominantly a disease of men more than forty years of age who live in congested industrial areas where air pollution is severe. Persistent cough, purulent sputum, and frequent bouts of bronchopulmonary infection with eventual expiratory obstruction terminate in respiratory failure and cor pulmonale (3). In the United States, chronic obstructive emphysema is likewise a disease chiefly affecting men more than forty. Respiratory failure and cor pulmonale terminate the illness; and an individualized form of air pollution, cigarette smoke, has been implicated (4). The most notable characteristics of obstructive emphysema are dyspnea, expiratory obstruction and increased functional residual lung volume; infection, cough, or exposure to air pollution is not essential.

The absence of *H. influenzae* from the sputum cultures of this group was in marked contrast to the frequency of isolation of this organism reported in chronic bronchitis. This complete absence was unexpected, and the possibility of technical error must be considered. Perhaps the use of other culture methods would have yielded different results. Although heated blood (chocolate) agar is often recommended as a culture medium for *H. influenzae*, the procedure used has been found satisfactory (22). Davis and associates followed 29 patients with pulmonary emphysema for more than twelve months and, although *H. influenzae* was isolated from the sputum, they were unable to correlate its appearance or disappearance with any change in status. Utilizing tetracycline, they were unable to confirm any benefit from chemoprophylaxis (23).

In addition to the effect of chemotherapy on the sputum, particular attention was paid in the present study to the subjective reaction of the patients to treatment and their objective responses as measured by gross ventilatory function tests. Most of the patients of both groups believed that they had improved, while pulmonary function tests demonstrated the opposite. This disparity stresses the importance of objective measurements in accurately evaluating different modes of therapy. A possible drawback to the use of pulmonary function tests is that they may not be sufficiently sensitive, and, in addition, patient cooperation is essential. A large number of tests were performed in the expectation that one procedure might prove more sensitive than another. This did not prove to be the case, and there is little reason to do more than one or two simple tests in such a study as this. The measurement of maximal expiratory flow rate by means of a Puffmeter has been found to be adequate for routine evaluation.

In conclusion, no evidence was found that erythromycin altered the course of chronic pulmonary emphysema during twelve weeks of administration. Similar negative results have been obtained with another antimicrobial drug given for a year (23). It is suggested that the physician treating this disease reserve the administration of antimicrobial drugs for the prompt treatment

of intercurrent bacterial respiratory infections. It has been noted in other conditions that the prolonged use of an antimicrobial drug may be attended by an actual increase in pulmonary infections, often with drug-resistant bacteria (24).

Summary

Twenty patients with chronic obstructive pulmonary emphysema were treated for three months with erythromycin or a placebo. By their own estimate most of the patients improved moderately during the period of treatment. Gross ventilatory measurements showed mild deterioration in almost all of the patients. There was no difference between the erythromycin- and placebo-treated cases when compared as whole groups or by individual pairs. Pneumococci disappeared from the sputum of patients receiving erythromycin, leaving a predominant flora of *N. catarrhalis*. Intercurrent respiratory infections occurred with the same frequency in both groups.

Sumario

La Administración de Antibióticos en el Enfisema Pulmonar Oclusivo Crónico. Estudio de Doce Semanas con Eritromicina

A 20 enfermos que tenían enfisema pulmonar oclusivo crónico se les trató durante tres meses con eritromicina o con un placebo. Conforme a su propio juicio, la mayor parte de los enfermos mejoraron moderadamente durante el período de tratamiento. Las mediciones de la ventilación cruzada revelaron leve deterioro en casi todos los sujetos. No hubo diferencia entre los tratados con eritromicina y los tratados con placebo al compararlos ya en grupos íntegros o en pares separados. Los neumococos desaparecieron del esputo de los enfermos que recibieron eritromicina, dejando una flora predominante de *N. catarrhalis*. Las infecciones respiratorias intercurrentes se presentaron con la misma frecuencia en ambos grupos.

Resume

Administration d'antibiotiques dans l'emphysème pulmonaire chronique obliterant. Etude d'un traitement de 12 semaines par l'erythromycine

Vingt malades présentant un emphysème pulmonaire chronique oblitérant ont été traités pendant trois mois avec de l'érythromycine ou un "placebo." La plupart des malades ont estimé qu'ils avaient été légèrement améliorés pendant le traitement. Des mesures sommaires de la ventilation ont montré une légère déterioration dans la plupart des cas. Aucune difference n'a été notée lors de la comparaison des deux groupes ou de personne à personne, entre les malades traités par l'érythromycine et ceux traités par le "placebo." Les pneumocoques ont disparus de l'expectoration des malades traités par l'érythromycine, laissant une flore où prédominait le *N. catarrhalis*. Les deux groupes ont présenté des infections respiratoires intercurrentes avec la même fréquence.

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THE EXPIRATORY VENTILAGRAM[1]

Application of Total and Timed Vital Capacities and Maximal Expiratory Flow Rate, as Obtained by a Bellows Apparatus, for Bedside and Office Use

GLENN E. HORTON AND SAMUEL PHILLIPS

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INTRODUCTION

The diagnosis, management, and therapy of patients with chronic pulmonary diseases and certain cardiac disorders may pose baffling problems to the physician. The diagnosis in the early stages of these conditions may be especially difficult. Frequently, the clinical findings, roentgenographic examinations, and other data yield imperceptible or insufficient information for proper management of the patient's problem. Physiologic tests have been developed recently to aid the clinician in this problem and they are as informative of the various functions of the lung as those similarly used in cardiac, hepatic, and renal disease (1). However, these pulmonary function tests in the past have required relatively elaborate equipment and especially trained personnel, so that their use was restricted mainly to special centers. Recently, some progress has been made in developing simpler methods which retain sufficient qualitative accuracy and are suitable for use in the office, clinic, or hospital ward.

It is the purpose of this paper to describe such a simplified method. A bellows apparatus was used to obtain total and timed vital capacities and also the maximal expiratory flow rate. These were recorded on a single graph, and the results were compared with those obtained with the conventional spirometer. Illustrative cases and practical clinical applications are presented briefly.

PROCEDURE

Methods: Use was made of an instrument which had been reported previously (2) and to which some minor improvements for better clinical application have since been made. This consists of a compact bellows vital capacity apparatus,[2] light in weight and small in bulk, containing a small motor-driven chart carrier. A pressure-sensitive chart has been placed on the latter, and a stylus is present to record the expiratory flow curve on the chart. The patients use a disposable cardboard mouthpiece which is placed over the chrome-plated taper on the front of the instrument connected to the bellows.

Operation of the apparatus is simple. An electrical cord from this compact instrument is plugged into a 100-volt, 60-cycle electric current, and the unit is ready for use in the office or at the bedside. By pushing a button located on the right side of the unit, the carriage containing the chart begins revolving. The patient placed in the standing position is then instructed to inhale as deeply as possible and, when the position of maximal inspiration is attained, the push button on the instrument is pressed and held down continuously. The patient is then asked to exhale smoothly and completely without force or haste until his expiration is complete. This is repeated until this value, or parameter, is maximal; or it is repeated until the values obtained agree within 100 ml. This is the observed *total vital capacity.* The predicted normal vital capacity is based on height, age, and sex and it can be calculated readily from an available regression formula (3) or obtained from previously prepared charts. The observed total vital capacity is then expressed as a percentage of the predicted normal vital capacity.

The *timed vital capacity* is obtained next. The procedure first should be explained to the patient. He should be shown how to make a forced rapid expiration after having attained the position of maximal inspiration. The largest volume of gas he can expire in one and in three seconds (*see* figure 1) is recorded and may be expressed either in per cent of the largest total vital capacity of the individual or in per cent of the largest total vital capacity for that person if he were normal. The latter figures are termed usually the "predicted" capacities. Because the predicted capacities are based on accumulated observations obtained with normal subjects, the total and timed actual vital capacities of a normal person will coincide usually with his predicted capacities. This is not true of patients with pulmonary disease, in which the differences between the actual and the predicted may form some objective bases for the measurement of disability.

Most writers express the timed vital capacity in terms of the actually observed vital capacity. However, there are occasionally times when it may

[1] From the Pulmonary Disease Service, Veterans Administration Teaching Group Hospital (Kennedy), Memphis 15, Tennessee.

[2] Vitalor®, McKesson Appliance Company, Toledo, Ohio.

be useful to express it, particularly the one-second expiratory capacity, as a per cent of the predicted total vital capacity, as will be shown in illustrative *Case 2*. The normal older patient should be able to expire at least 65 per cent of his total vital capacity in the first second and at least 90 per cent by the third second; the young adult usually will exhale faster, while a child may be able to expire 90 per cent of his total vital capacity within the first second.

A brief estimate of the *maximal expiratory flow rate* (*MEFR*) is then made. This measurement is

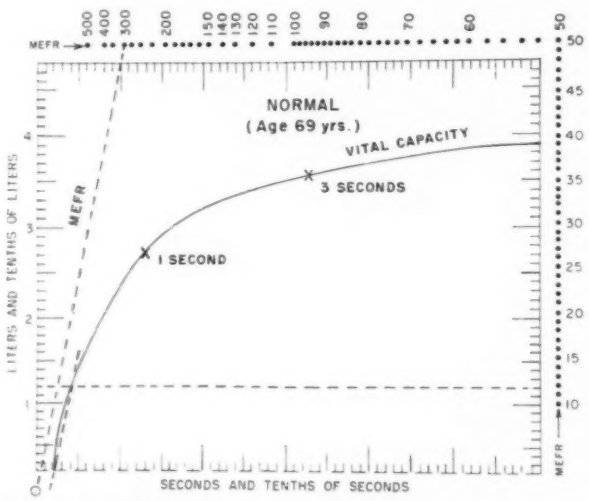

FIG. 1. *Case 1*. Graphic chart showing a normal ventilatory pattern. The total and timed vital capacities are within normal limits for the patient's age. The third parameter, the maximal expiratory flow rate (MEFR), is 300 liters which is also normal. (*See* text for simple and rapid method employed).

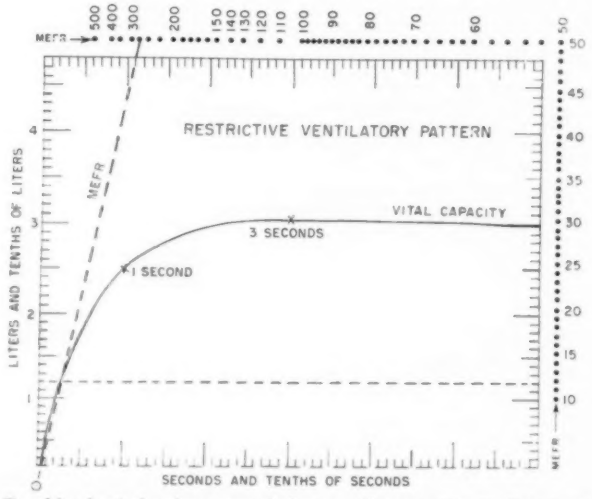

FIG. 2. *Case 2*. Graphic chart showing a restrictive ventilatory pattern. The *expiratory ventilagram* shows a decrease in the total vital capacity (73 per cent) of predicted normal but a normal actual timed vital capacity and expiration time and close to normal maximal expiratory flow rate (MEFR).

calculated from the expiration curve, utilizing the time required for the subject to expire one liter of air, beginning at 200 ml. and ending at 1,200 ml. It is expressed in liters per minute. A straight line is drawn on the chart from the point where the graph begins at the horizontal baseline to the point where the graph crosses the 1,200 ml. line. Then a second straight line (*see* figure 1) is drawn parallel to the first, starting from the dot within the small circle which is located at the lower left-hand corner of the chart and extending to the upper or outer edge of the chart. Obviously, if these described three points fall in one line as may be seen in figure 2, the second parallel line will coincide with the first. It is possible by this method to read the MEFR directly from the chart, as it is the value printed at that point where the extended straight line crosses the MEFR dots on the upper or outer edge of the chart. The derivation and calibration of the MEFR referred to here have been described previously (2). The minimal normal value is considered at present to be 300 liters per minute, or perhaps slightly less. Characteristically, the MEFR is markedly lowered in "obstructive" bronchopulmonary disease, and usually only moderately lowered in "restrictive" respiratory conditions or in left ventricular failure

It should be mentioned that all determinations tabulated in this paper which were obtained with the bellows apparatus were performed and calculated by the senior writer, and took usually five minutes or less for each patient.

The results of the total and timed vital capacities obtained with the bellows apparatus were then compared with the values obtained with the 9-liter spirometer.[3] Operation of this latter standard instrument is discussed in detail elsewhere (3). An attempt was made to perform these comparison tests on the same day on each patient in order to try to ensure minimal changes in the clinical state of the patient and also to avoid changes in the temperature of the hospital rooms. The spirographic tracings were done either by the technician or the physician, with all tracings carefully inspected by the physician. If it was believed that the patient did not perform maximally, the tests were repeated. This was particularly important in *emphysematous* patients.

Results

The marked agreement between the figures for the total and the three-second timed vital capacities obtained with the portable bellows apparatus as compared with those obtained with the 9-liter spirometer is shown in table 1.

The mean difference between the values for the total vital capacity as obtained by the two methods was 0.05 liters, with a standard deviation of 0.13 liters and a 0.95 confidence interval of +0.31 liters to −0.21 liters. The mean difference between values for the three-second vital capacity was 0.38 per cent, with a standard deviation of 2.0 per cent and a 0.95 confidence interval of +4.38 to −3.62 per cent. The one-second timed vital capacity and maximal expiratory flow rate values could not be compared satisfactorily for statistical purposes because of the lack of the necessary kymographic speed[4] and calibration required for this measurement, which were not available on the conventional spirometer used in this study.

Comment: Physiologic tests of pulmonary function comprise two general groups. The first relates to the bellows action of the chest and lungs for the movement of air in and out of the alveoli (*ventilation*) and the *distribution* of the inspired gas throughout the alveoli. The second relates to the *diffusion* of gas across the alveolar capillary membranes, to and from the blood, into the *perfusing* pulmonary capillaries of the lung. The three parameters presented in this paper are limited principally to ventilation and may be referred to as the *expiratory ventilagram*. This term itself is not original with the writers (4). Generalized disease of the lung, particularly the ventilatory restrictive and obstructive types, may significantly alter the pulmonary function, and yet only minimal abnormalities may be exhibited on roentgenographic examination (1).

There is fairly general agreement among investigators on the "basic" equipment and methods needed to test pulmonary function (5). The minimal evaluation includes a total and a timed vital capacity. The measurement of the total vital capacity without the estimation of the rate of expiratory air flow is relatively valueless. In the office or at the bedside, this can be estimated by observing the rate at which the patient expires into the apparatus used for measuring the vital capacity. A more accurate measurement of the maximal rate of expiratory air flow can be made by an inspection of the actual expiration curve made during maximal effort, whether on the spirogram or on the graph, as presented in the method described in this paper.

As stated by Comroe and associates (1):

> If vital capacity and maximal volume flow of air are both normal, over-all mechanical function is good and the cause of dyspnea must be sought elsewhere. If vital capacity is reduced greatly,

[3] Nine-liter Respirometer®, W. E. Collins Co., Boston 15, Massachusetts.

[4] Now available.

TABLE 1

TABULATION OF RESULTS ON BELLOWS APPARATUS (A)* COMPARED WITH CONVENTIONAL 9-LITER SPIROMETER (B)†

(See text for statistical correlation and further discussion)

Subject	Total Vital Capacity (in liters)		Timed Vital Capacity				Major Pulmonary Pathophysiology
	A*	B†	First A	Second B	Third A	Second B	
1. R. G.	3.90	3.81	70	64	90	89	Normal (age 69)
2. H. P.	3.00	2.94	83	73	100	97	Sarcoidosis
3. P. M.	2.76	2.84	31	44	69	71	Emphysema
4. G. H.	4.80	4.83	73	67	96	94	Normal
5. D. L.	4.78	4.83	64	66	88	90	Apical fibrosis
6. T. L.	3.49	3.62	57	54	86	88	Bronchial asthma
7. C. R.	4.02	3.88	64	58	90	88	Apical bronchiectasis
8. J. C.	3.42	3.27	59	60	90	90	Fibrosis
9. A. M.	4.80	4.77	85	80	100	99	Apical histoplasmosis
10. R. R.	2.68	2.87	64	55	88	89	Tumor
11. A. B.	2.70	2.65	63	62	86	84	Fibrosis
12. R. O.	3.15	3.30	76	74	95	94	Fibrosis
13. W. M.	1.94	1.84	39	36	67	67	Myeloma
14. F. W.	2.75	2.92	36	22	62	61	Emphysema
15. L. S.	2.06	2.07	48	46	83	81	Emphysema
16. F. W.	2.21	2.16	56	56	85	86	Histoplasmosis
17. S. D.	3.51	3.57	54	50	78	78	Blastomycosis
18. B. K.	2.68	2.69	35	24	58	58	Emphysema
19. B. R.	3.26	3.19	58	47	81	84	Emphysema
20. J. W.	2.46	2.40	59	69	94	96	Congestive failure
21. D. T.	1.71	1.74	41	39	71	70	Emphysema
22. J. L.	2.90	2.85	34	33	68	61	Emphysema
23. E. H.	2.50	2.49	68	67	91	91	Sarcoidosis
24. F. S.	1.68	1.66	39	33	63	61	Emphysema
25. L. S.	2.88	2.86	52	51	77	78	Emphysema
26. C. R.	4.37	3.87	80	84	96	97	Apical fibrosis

* Vitalator®, McKesson Appliance Co., Toledo, Ohio.

† Nine-liter Respirometer®, W. E. Collins Co., Boston, Massachusetts.

but maximal volume flow of air is nearly normal, a restrictive lesion probably exists. If vital capacity is nearly normal, but the maximal volume flow of air is reduced greatly, an obstructive lesion probably exists. Furthermore, these tests, performed before and again after the administration of bronchodilator drugs, are often valuable in determining whether the patient has reversible bronchial obstruction; in this way they are very helpful both in diagnosis and in directing therapy.

The maximal volume flow of air is interpreted as corresponding for practical purposes with the maximal expiratory flow rate and the actual timed vital capacity. It might also have been termed the maximal rate of flow.

Illustrative Cases

Normal Ventilatory Pattern:

Case 1. R. G., a 69-year-old white male with a mild nervous disorder, had no known respiratory disease. The total and timed vital capacities (figure 1)[5] were normal as predicted for his age, height, and sex. Inspection of the expiratory curve revealed a probable normal contour for his age. The maximal expiratory flow rate was also normal, with an observed value of 300 liters. These findings were closely correlated with the findings on the 9-liter spirometer (*see* table 1). Further tests of pulmonary ventilation revealed that the maximal breathing capacity was normal in value and performance (3). The residual volume was normal as well as the ratio of the residual volume to the total lung capacity (6). The index of intrapulmonary distribution of the inspired air (7) was also normal.

This patient demonstrates the normal values found with the ventilatory physiologic tests of pulmonary function. The present experience indi-

[5] Reproduced without central grid-lines for publication reproduction purposes.

cates that, if the three parameters, the *expiratory ventilagram*, as obtained with the bellows apparatus discussed above, are normal, there is a reasonable likelihood that the maximal breathing capacity, the residual volume, and the index of intrapulmonary distribution of inspired air will be found close to normal limits. This applies only to *normal* subjects. The implication is that these parameters may help to serve as a useful screening and qualitative method of differentiating normal subjects grossly from those with early restrictive and obstructive lung disease or with certain types of heart disease, from patients with certain emotional disorders without organic disease, particularly those in whom the *exertional dyspnea* or breathlessness is out of proportion to the other clinical findings.

Restrictive Ventilatory Pattern:

Case 2. H. P., a 39-year-old Negro male with generalized sarcoidosis, had the characteristic clinical, roentgenographic, and laboratory findings. The diagnosis was confirmed on lymph node biopsy. The chest roentgenogram revealed bilateral, diffuse, fine, granular infiltrates. Exertional dyspnea was the chief respiratory complaint, which improved after adrenocortical steroid therapy. This clinical improvement was confirmed on the objective physiologic tests of pulmonary ventilation. While the patient was on maintenance steroid therapy, the total vital capacity (figure 2) on the bellows apparatus revealed 3.0 liters or 73 per cent of the predicted normal. However, the timed vital capacity revealed that the patient was able to expire 83 per cent of his *actual total vital capacity* in the first second, but only 62 per cent of his *predicted total vital capacity*, because he had a significantly reduced total vital capacity as compared with normal. He was able to expire his actual total capacity in three seconds. The maximal expiratory flow rate was almost normal at 270 liters. These findings were checked and corroborated by conventional spirometry (*see* table 1).

This patient showed the characteristic restrictive pattern of ventilation impairment as seen in pulmonary fibrosis, such as may be associated with sarcoidosis. Such findings indicated a need for further investigation. The one-second expiratory capacity here expressed as per cent of predicted total vital capacity correlated very closely with the maximal breathing capacity, similarly expressed. This particular correlation in both normal subjects and patients with pulmonary disease has been reported previously by Gaensler (8). The performance of maximal breathing capacity showed no evidence of expiratory airway obstruction. The residual volume was normal as well as the residual volume and total lung capacity ratio and the index of intrapulmonary distribution of inspired gas. Arterial gas studies revealed arterial oxygen unsaturation at rest, and this was worsened by exercise but completely overcome by the administration of 100 per cent oxygen. In this patient, the ventilatory tests were compatible with a restrictive ventilatory pattern, and the arterial gas studies suggested a diffusion impairment also. Here, the basic abnormalities demonstrated on the *expiratory ventilagram* prompted further investigation, which revealed additional information of the extent and the mechanism altering the pulmonary physiology.

Obstructive Ventilatory Insufficiency:

Case 3. P. M., a 51-year-old white male plumber with a history of cough and sputum production, had clinical and fluoroscopic findings compatible with those of pulmonary emphysema. The total vital capacity was 2.76 liters, or 74 per cent of predicted normal, with a marked prolonged expiration time (figure 3). The timed vital capacity indicated that 31 per cent was expired in the first second and 69 per cent in three seconds. The maximal expiratory flow rate was markedly reduced, 38 liters. These findings were also corroborated with the conventional spirometer (*see* table 1).

Again, the initially observed ventilatory findings prompted further investigation employing other tests of pulmonary ventilation. The observed value of the maximal breathing capacity was markedly reduced. The performance of the maximal breathing capacity showed employment of the *inspiratory position*, as seen in *expiratory airway obstruction*. The residual volume was increased, and the ratio of the residual volume to total lung capacity was increased to 43 per cent. The index of intrapulmonary distribution of inspired air was prolonged. Here, the three parameters measured on the bellows apparatus were abnormal, and further clarification was obtained with additional tests of pulmonary ventilation.

The altered physiologic function was that of expiratory airway obstruction with retention of air, and a poor distribution of inspired air. The results were little altered by bronchodilator therapy. The over-all results were compatible with that of obstructive emphysema and offered a reasonable assessment of the nature of the altered physiology.

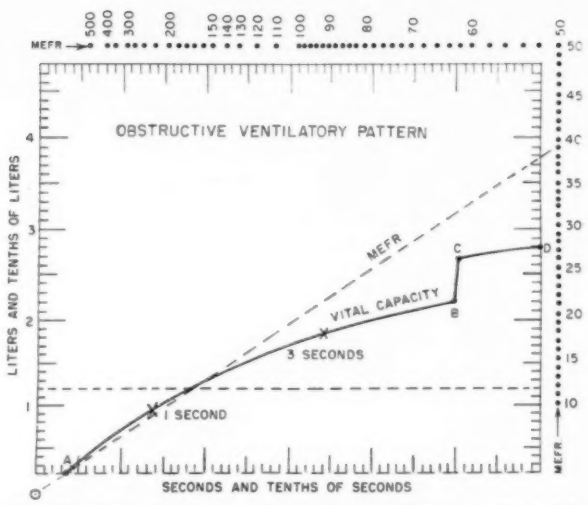

FIG. 3. *Case 3.* Graphic chart showing an obstructive ventilatory pattern. Observed parameters are discussed in the text. Because of the prolonged expiration time (10 seconds in this patient) it was necessary to improvise slightly. The motor was stopped at point B and the patient continued to breathe in the bellows (recording stylus continued to move vertically). Then the motor was turned on at point C after several seconds. A to B and C to D measure volume and time and B to C volume only. From A to B the timed vital capacity volume is determined in first and third seconds, and from A to D the total vital capacity is arrived at.

GENERAL COMMENT

The *expiratory ventilagram* also may be of value in the management of cardiovascular disease. It is well known that congestion of the pulmonary vascular bed in left-sided heart failure causes a decrease in the total vital capacity. However, little attention has been paid to the associated changes in the expiratory flow rate until relatively recently, particularly through the work of Gaensler (8), Ebert (9), and Franklin (10, 11) and their co-workers. The latter referred to the total and timed vital capacity as the "expirogram." This, however, does not include a maximal expiratory flow rate parameter.

Actually, pulmonary dyspnea of cardiac origin may arise from a combination of faulty ventilation, compliance, and diffusion (12). There is the *acute* or *recent* left-sided type of congestive failure which is usually characterized by a restrictive ventilatory pattern similar to that in figure 2, i.e., rapid expiration in time with diminished total vital capacity. Then, there is the type observed in *chronic* left-sided heart failure or in *long-standing* pulmonary congestion, as seen in the patient with mitral stenosis, which may show a pattern similar to the first but with more terminal sloping of the expiration flow curve. The value of pulmonary function studies, especially the vital capacity parameters, in evaluating the symptom of *dyspnea* in patients with mitral stenosis has been stressed recently by Friedman and associates (13). The third pattern occurs in patients with *chronic pulmonary disease* and resulting heart failure which shows a slowing throughout the expiratory curve. This latter prolonged expiratory curve is typical of those seen in pulmonary emphysema without clinical evidence of cor pulmonale. Therefore, right-sided heart failure is not detectable by this means directly (11). However, pulmonary function studies may offer inferential evidence as to the actual extracardiac origin of the right ventricular involvement (12). After treatment, the vital capacity increases in this type of pattern but the expiratory flow rate remains low.

* * *

The method described in this paper is not meant to replace the more quantitative parameters of pulmonary function testing as carried out in excellent laboratories in this country and

abroad, but serves as a qualitative index, or as a guide, to the nature of the disease. However, tests of pulmonary functions have limitations (14), as do all tests used in medicine. As pointed out by Curtis (15) and applied here: "It must be emphasized that all pulmonary function tests are adjunctive to the history, physical examination, chest roentgenogram, and careful fluoroscopy. Together and properly interpreted, these provide the means of accurately evaluating the patient with pulmonary disease."

Summary

A practical and comparatively simple method has been presented of testing the pulmonary function of a patient in the physician's office or on the hospital ward. The total and timed vital capacities are recorded on a permanent graph, together with the expiratory flow curve and the maximal expiratory flow rate. These parameters have been referred to as the *expiratory ventilagram*. The accuracy of the instrument is comparable with that of the conventional spirometer, particularly in regard to the total and three-second timed vital capacities. Clinical application of these measurements is discussed, and their use on a wider scale is suggested for more effective patient care.

Sumario

El Ventilagrama Espiratorio

Preséntase aquí un método práctico y comparativamente sencillo para determinar la función pulmonar de un enfermo en el consultorio del médico o la sala de un hospital. Las capacidades vitales total y medida en tiempo se registran en una gráfica permanente, junto con la curva de la corriente espiratoria y el coeficiente de la corriente espiratoria máxima. Estos parámetros se denominan *ventilagrama espiratorio*. La exactitud del instrumento es comparable con la del espirómetro corriente, en particular con respecto a las capacidades vitales total y medida de 3 segundos. Se discute la aplicación clínica de estas mediciones y se aboga por su empleo en escala más amplia para suministrar asistencia más eficaz al enfermo.

Resume

Le ventilogramme expiratoire

Une méthode pratique et relativement simple a été présentée pour contrôler le fonctionnement pulmonaire du malade au cabinet du médecin ou à l'hôpital. La capacité vitale totale et celle pour une durée déterminée est enregistrée sur un diagramme permanent, ainsi que la courbe du volume expiratoire et le taux du volume expiratoire maximum. Ces paramètres sont appelés: ventilogramme expiratoire. La précision de l'instrument est comparable à celle du spiromètre ordinaire, notamment en ce qui concerne la capacité vitale totale et la capacité 3-secondes. L'application clinique de ces mesures est discutée et leur emploi sur une plus grande échelle est suggéré pour assurer un traitement plus efficace des malades.

Acknowledgment

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Case Reports

RHEUMATOID LUNG DISEASE[1]

Report of a Case Which Developed in Childhood

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INTRODUCTION

Rheumatoid lung disease was first described by Ellman and Ball (1) in 1948; numerous cases have been reported since then. This literature was recently reviewed by Rubin (2) who added four more cases of his own. However, there have been comparatively few reports in which autopsy examination was included. The reason for reporting another case is that lung changes were first noted in this patient at the age of six years, with progressive deterioration resulting in death at the age of fifteen years. Furthermore, the lung pathology in the present case differs to some extent from that previously reported in rheumatoid lung disease.

CASE REPORT

The patient, *H. F. H.* (No. 855929), a thirteen-year-old female, was first admitted to the hospital in November, 1956. At six weeks of age, this child had whooping cough and pneumonia. During the next eight years she had ten attacks of pneumonia, which necessitated her admission to the hospital on each occasion. At ten years she had an episode of acute arthritis diagnosed as rheumatic fever. Following this episode, she had gradually progressive changes in the joints of the hands and feet with occasional shoulder and knee pains. Also during the three-year period prior to her first admission to this hospital, she developed progressive dyspnea with the simultaneous development of cyanosis and clubbing.

Her grandmother and aunt both had pulmonary tuberculosis, but the parents and one sibling gave no relevant history.

On examination, the patient was markedly cyanosed at rest, with gross clubbing of fingers and toes. The trachea was central; the chest moved moderately well; there was normal resonance. The breath sounds were vesicular, but there were generalized moist, medium rales over the entire right lung and over the lower half of the left lung. She had no palpable nodes. Liver and spleen were not palpable. The interphalangeal joints were swollen. The elbows, knees, ankles, and fingers and toes were slightly swollen with some limitation of movement. She was seen in the Arthritis Division of the hospital and the diagnosis of inactive Still's disease with superadded pulmonary osteoarthropathy was made (figures 1 and 2).

Laboratory examinations showed that she had a hemoglobin of 13.8, an erythrocyte count of 5 million per cu. mm., a leukocyte count of 5,150 per cu. mm., with 75 per cent polymorphonuclears, 3 per cent eosinophils, 21 per cent lymphocytes, 1 per cent monocytes. A serologic test for syphilis was negative; sedimentation rate was 28 mm. in one hour. A routine urine examination was negative. A sweat chloride test was 37 mg., which is within normal limits for this laboratory. Serum electrophoresis showed a total protein of 6.9 gm. per 100 ml., with albumin, 37 per cent; gamma-globulin, 17 per cent; beta-globulin, 20 per cent; alpha-2, 19 per cent; and alpha-1, 8 per cent. A test for lupus erythematosus was negative.

The vital capacity was 1,190 cc. (predicted normal 2,970); the 3-second vital capacity was 1,170 cc.; the maximal breathing capacity was 70 liters per minute (predicted normal 90 liters per minute). Resting arterial blood studies gave an oxygen saturation of 92 per cent; carbon dioxide tension of 39 mm., carbon dioxide content of 19.8 mEq., and pH of 7.36.

Chest roentgenograms taken in 1949, at six years of age, showed scattered areas of infiltration in both midlung fields. Subsequent films showed a gradual increase in the infiltration so that at the time of the child's admission to this hospital at thirteen, there were extensive, diffuse, bilateral fibrotic changes of marked degree (figure 3). Hilar node enlargement, particularly well demonstrated in the azygous area was observed. Roentgenograms of the hands showed early bone atrophy, with soft tissue swelling proximally, and clubbing of the terminal phalanges, but no destructive punched-out areas of bone were demonstrated.

Bronchoscopy showed slight reddening of the bronchial mucosa on both sides with minimal secretions. Bronchograms were normal. A left scalene node biopsy was reported as a hyperreactive lymph node. A left lung biopsy was performed by open thoracotomy. At operation the lung was studded with small grayish avascular nodules which were palpable on the surface of the visceral pleura. The margins of the lung were avas-

[1] From the Division of Pulmonary Diseases and the Department of General Surgery, Henry Ford Hospital, Detroit, Michigan.

FIG. 1 AND 2. The gross clubbing and deformity of the hands and feet at the time of the patient's first admission to the hospital.

cular and grayish looking. The gross appearance was that of a granuloma. A wedge of the lingula was removed, and the biopsy report was pulmonary fibrosis and thickened pleura with evidence of chronic inflammation (figure 4).

The final diagnosis was rheumatoid arthritis with pulmonary osteoarthropathy and pulmonary fibrosis of uncertain etiology but probably related to the patient's rheumatoid arthritis. She was started on prednisolone, 5 mg. four times a day, and over the next week this dose was reduced to 5 mg. twice daily. She was discharged and continued with slight variations on this dosage for the next five months, during which time her joints gave her little trouble; but there was no improvement in her exercise tolerance, in the physical signs, or in the chest films. In August the patient was restarted on prednisolone, 1 mg. three times daily, and continued on this for the next six months. Throughout this time her cough and exercise tolerance worsened, while her weight dropped from 113 to 98 pounds.

By February, 1958, her weight was only 89 pounds, and she had become severely dyspneic and cyanosed. She was therefore started on triamcinolone, 4 mg. four times a day, and this dose was gradually reduced to 2 mg. three times a day. Despite this, her weight dropped to 84 pounds, and early in April, 1958, she was started on intermittent low-flow oxygen therapy at home. This was taken by anterior nares nasal catheter during sleep and periodically during the day. For two months there was definite improvement, during which time she slept better and had more energy during the day, while the morning headache, which had been severe previous to this, disappeared. However, this improvement was short-lived and by August, 1958, she was completely incapacitated.

The patient's final admission to the hospital was in August, 1958. On physical examination she was deeply cyanosed, dyspneic at rest, had very poor chest expansion, and obviously a poor tidal volume. There were scattered rales throughout

FIG. 3. Roentgenogram showing the diffuse fibrotic changes throughout both lungs.

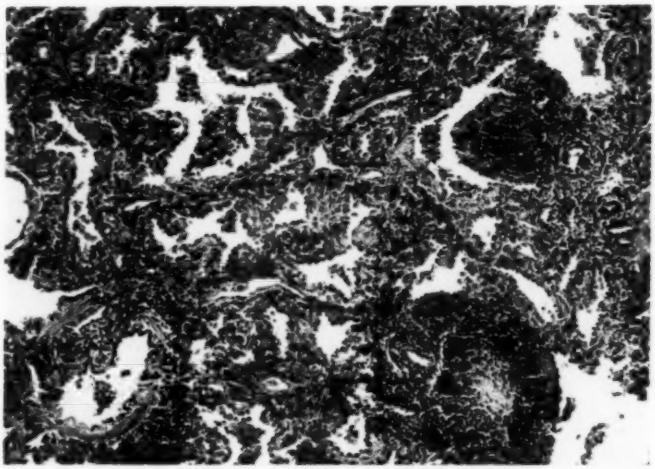

FIG. 4. Photomicrograph from biopsy specimen. Note dense lymphocytic infiltrate, "septal cell" hyperplasia, and diffuse thickening of alveolar septae. A respiratory bronchiole is present in lower left corner. (Hematoxylin and eosin stain, × 150)

the chest but no wheezing. Her hemoglobin was 14.8, routine urine examination showed a faint trace of albumin, and resting arterial blood studies at this time showed an oxygen saturation of 39 per cent with carbon dioxide tension of 56 mm., a carbon dioxide content of 21.4 mEq., and a pH of 7.25. A tracheotomy was done, and one hour following this her arterial blood showed an oxygen saturation of 64 per cent, carbon dioxide tension of 52 mm., carbon dioxide content of 19.3 mEq., and a pH of 7.23. She was then given oxygen, but her condition worsened. She had a convulsion, became unconscious and did not recover, and died some sixteen hours following the operation. An autopsy was performed.

Summary of pathologic findings: Necropsy was performed eight hours after death. The body weighed 83 pounds and measured 150 cm. in length. A vertical midline tracheostomy was patent, and the cannula was found to be entering the trachea.

Marked cyanosis and clubbing of all digits of both the upper and lower extremities were noted. No evidence of lymphadenopathy, joint deformities, or swellings or palpable subcutaneous nodules were found. The stomach was markedly dilated and distended, and contained 1,700 ml. of green fluid. It was believed that the severity of the gastric dilatation could have increased the pulmonary difficulty and been a factor in her death.

FIG. 5. Transverse section of upper lobe of right lung. Note cysts of various sizes and intracystic fibrosis.

The pleural cavities contained 5 to 10 ml. of serous fluid. Multiple slate-gray fibrous adhesions were found almost completely obliterating the left pleural cavity. Some of these were also attached to the pericardium. Between the anterior and the lateral surfaces of the right lower lobe and chest wall there were multiple shaggy fibrinous and fibrous adhesions. The lungs weighed 1,250 gm. and were slate-gray and rubbery. They were practically noncrepitant. The bronchi and pulmonary vessels were within normal limits. Particularly striking was the honeycomb appearance of the cut surface. The cysts varied from less than 1 mm. to 1 cm. in diameter, did not contain fluid, and had smooth slate-gray linings (figure 5); these changes were most striking in the dependent and peripheral portions of the right and left upper lobes. The central portion of the lungs showed areas of consolidation. Lymph nodes in the hilar area measured 1 to 1.5 cm.

Microscopically, the larger lung cysts were lined by cuboidal epithelium and occasionally were continuous with small bronchioles (figure 6). The alveolar septa were markedly thickened due to infiltration with lymphocytes, occasional plasma cells, and fibroblasts. Many of the alveoli contained proteinaceous material and a varying number of lymphocytes. Numerous sections showed areas of lymphoid infiltration which was

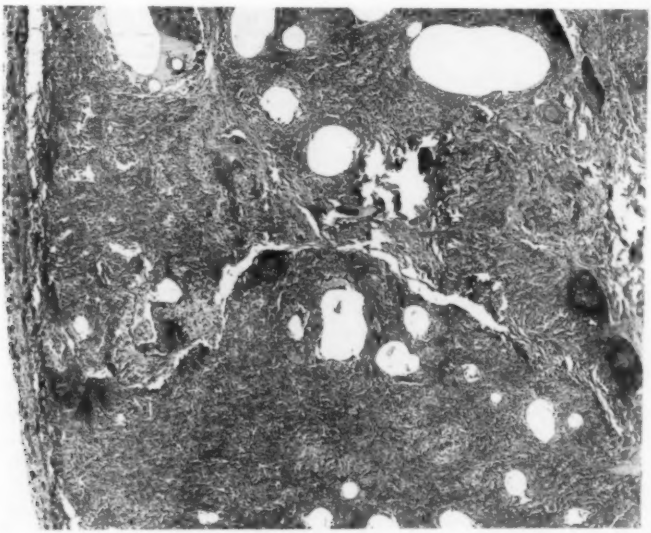

FIG. 6. Low-power view of lung after necropsy. Many of the cysts in the central portion of the photograph show a communication with small bronchioles. Note nodular accumulations of lymphocytes. (Hematoxylin and eosin stain, × 22)

occasionally nodular. This was particularly prominent in the surgical specimen (*see* figure 1). There were a few giant cells, but no distinct granulomas were found. The pleura was markedly fibrotic in areas, with a moderate mononuclear cell infiltrate.

The synovia of the knee and hip joints showed changes of rheumatoid arthritis. There was subendocardial fibrosis and hypertrophy of the myocardium but no evidence of rheumatic heart disease. The liver showed evidence of chronic passive congestion. Enlarged nodes were found in the mesentery, para-esophageal, and retroperitoneal areas which microscopically showed sinous histiocytosis with occasional centers of necrosis.

There were no significant vascular changes in the lungs or in any other organ.

Discussion

Leys and Swift (3) reported a child of six years with rheumatoid arthritis in whom transient lung changes occurred which they thought represented rheumatoid lung disease. However, no biopsy was obtained, and subsequently the chest roentgenogram cleared completely.

The patient described in the present paper is the youngest case of rheumatoid lung disease so far reported. She also represents the ninth case with autopsy findings (1, 4–6). Although several cases of lung biopsy have been reported, the pathology of this entity is not yet clearly defined. While Price and Skelton (7) considered arteritis of major importance, in the cases reported by Ellman and Ball (1), Christie (4), and in the present case, the vascular changes were insignificant.

The lack of distinct granuloma formation makes this case different from those reported by Christie (4), Bennett and associates (8), Gruenwald (9), and Gresham and Kellaway (10), who reported granuloma as part of the picture of the rheumatoid lung. The lungs do show evidence of severe pulmonary fibrosis and marked cystic changes. In 1948, Hart (11) reported a case of rheumatoid lung which at necropsy six years later (12) showed evidence of diffuse cystic changes which were believed to be more compatible with cystic disease of the lungs; the rheumatoid arthritis was considered to be coincidental. These writers therefore withdrew their case as an example of pulmonary rheumatoid disease. However, the changes described in the present case differ from those described by Hart and McKenzie and by Gresham and Kellaway, as the cystic spaces were lined by cuboidal epithelium, the lungs were not emphysematous, and little normal lung parenchyma was seen in the intercystic areas.

Summary

A case of rheumatoid lung disease is described in which roentgenographic abnormality was first noted at the age of six years, and death from pulmonary insufficiency resulted at the age of fifteen. At autopsy the lungs were markedly cystic but not emphysematous. There was extensive thickening of the alveolar septae. There were no distinct granulomas in any of the viscera. These changes are compared with the findings described in previously reported cases examined at autopsy.

Sumario

Neumopatía Reumatoidea. Presentación de un Caso Aparecido en la Niñez

Se describe un caso de afección reumatoidea del pulmón en el cual se notó primera vez anomalía radiográfica a la edad de seis años y sobrevino la muerte por insuficiencia pulmonar a la edad de quince años. En la autopsia, los pulmones estaban notablemente quísticos, pero no enfisematosos. Había extenso engrosamiento de los tabiques alveolares. No había granulomas bien definidos en ninguna de las vísceras. Se comparan estas alteraciones con los hallazgos descritos en los casos comunicados anteriormente y examinados en la autopsia.

Resume

Maladie pulmonaire rhumatismale. Rapport d'un cas chez l'enfant

Un cas de maladie pulmonaire rhumatismale est décrit dans lequel l'anomalie radiographique a été observée pour la première fois à l'âge de six ans; la mort est survenue à l'âge de 15 ans, par suite d'insuffisance pulmonaire. A l'autopsie les poumons présentaient de nombreux kystes mais pas de lésions d'emphysème. Un épaississement considérable des cloisons alvéolaires fut également constaté. Il n'y avait pas de lésion granulomateuse nette dans aucun des viscères. Ces modifications sont comparées à celles qui ont été décrites dans les cas examinés à l'autopsie et signalés antérieurement.

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PULMONARY DISEASE DUE TO ATYPICAL TUBERCLE BACILLI[1]

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Introduction

In recent years much attention has been given to the problem of pulmonary disease associated with acid-fast bacilli that differ in certain biologic aspects from *Mycobacterium tuberculosis*. The number of reports on this subject has shown a marked increase since the introduction of specific chemotherapeutic agents for the treatment of tuberculosis. It seems likely that more frequent recognition of the disease is due to the greater use of cultural methods for demonstrating tubercle bacilli and to a more critical appraisal of the cultural growth than was customary before the era of drug therapy.

In 1935, Pinner (1) pointed out that chromogenic acid-fast bacilli could be found in various human materials in association with disease, and emphasized the need for careful study in diagnostic procedures. Later, Baldwin (2), Feldman and associates (3), and Cory (4), reported disease associated with acid-fast bacilli that differed from tubercle bacilli. Since 1953, a series of reports (5–11) has appeared describing other cases of pulmonary disease associated with "atypical acid-fast organisms." These later papers, in an attempt to establish some sort of classification, describe more thoroughly the bacteriologic properties of the bacilli and provide data on their chromogenicity and capacity to produce disease in animals.

It is obviously important for physicians treating chronic pulmonary disease to be aware of the problem of disease caused by or associated with acid-fast organisms other than *M. tuberculosis* so that errors of diagnosis and treatment can be avoided. For this reason it was considered worth while to review the experiences concerning this problem at the Ray Brook State Tuberculosis Hospital in order to acquire information on the incidence, bacteriologic aspects, character of disease, treatment, and end results. In the past five years there have been 9 patients from whose sputum atypical tubercle bacilli were found repeatedly in sufficient numbers to be considered related to the disease process in the lungs. The total number of admissions was 1,700, but it must be pointed out that a majority of the patients were transferred from other hospitals and, therefore, were a selected group.

[1] From the Ray Brook State Tuberculosis Hospital, Ray Brook, New York.

Case Reports

Case 1. H. L., a 50-year-old policeman, was admitted to the hospital on March 19, 1953. Chest roentgenograms showed mottled densities above the second left and right anterior ribs, with a cavity at the right apex measuring 2 by 3.5 cm. Sputum was positive microscopically and by culture for tubercle bacilli. The patient was started on streptomycin, para-aminosalicylic acid (PAS), and isoniazid therapy and continued to November, 1953. Tubercle bacilli were susceptible[2] to streptomycin, PAS, and isoniazid. Sputum converted to "negative" in July, 1953, and continued so until May 21, 1954, when the first of a series of positive cultures appeared and have continued to appear at intermittent intervals. These, unlike the former cultures, proved to be atypical photochromogenic tubercle bacilli which did not produce disease in guinea pigs nor did the guinea pigs react to Old Tuberculin (OT). The bacilli were non-cord-formers. Susceptibility tests revealed the bacilli to be resistant to streptomycin and to PAS, but susceptible to isoniazid. The patient was discharged on August 4, 1955. Three sputum cultures in September, 1956, contained atypical tubercle bacilli, but two subsequent cultures in 1957, and two in 1958, were negative. His last chest roentgenogram on August 11, 1958, showed no change.

In reviewing this patient's problem, the evidence indicates that tubercle bacilli were present early in his illness, and that later atypical bacilli began to appear at occasional intervals and persisted over a period of two years.

Case 2. J. C., a 60-year-old janitor, was admitted March 19, 1954. A chest roentgenogram in November, 1953, had revealed extensive disease

[2] Routine drug susceptibility was determined by inoculating the patient's tubercle bacilli on egg medium (Hohn) containing 10 γ of streptomycin per ml., 10 γ of PAS per ml., and 1 and 5 γ of isoniazid per ml. No growth in the presence of a drug was considered evidence of susceptibility. Growth in the drug tubes was compared with that on the control tube without drugs to indicate degree of resistance.

in contrast to minimal abnormalities present in the last previous roentgenogram of March, 1948. Admission chest roentgenograms showed linear and mottled densities and a pneumothorax on the left. The right lung showed linear and mottled densities through the upper half with an apical cavity measuring 2.25 by 4.50 cm. An intradermal skin test with 1:100 OT was positive. Sputum was positive microscopically for acid-fast bacilli, and the patient was started on streptomycin, PAS, and isoniazid. The bacilli grew rapidly at 37°C. and were photochromogenic. They were partially resistant to streptomycin, resistant to PAS, and susceptible to isoniazid. They were also resistant to penicillin, chlortetracycline, and oxytetracycline. Streptomycin, PAS, and isoniazid were continued, and roentgenograms showed slow improvement; the evidence of a definite cavity disappeared, but numerous emphysematous bullae persisted. Resistance to isoniazid was demonstrated in a strain of bacilli isolated from the sputum on May 20, 1954. Similar acid-fast bacilli were found consistently through June 21, 1954, and the patient's sputum has been negative for acid-fast bacilli on 34 microscopic examinations and cultures to date. Inoculation of the organisms into guinea pigs failed to produce disease, and the tuberculin sensitivity of the guinea pigs was very low. The organisms did not form cords in liquid medium. The patient was discharged on July 5, 1956, and has continued to be well.

In summarizing this patient's problem, it is noted that at no time was *M. tuberculosis* found. The patient's clinical response was excellent and he is now considered well.

Case 3. M. M., a 55-year-old truckman, was admitted to Ray Brook on January 20, 1955. A chest roentgenogram in October, 1953, showed massive densities extending from both hila into the bases of the lungs. In August, 1954, the patient was admitted to a hospital for study and, following a demonstration in the sputum of acid-fast bacilli (subsequently shown to be atypical tubercle bacilli), he was started on streptomycin and PAS which were continued until his discharge from the hospital on October 23, 1954. At admission here, his symptoms were limited to a productive cough. Careful inquiry revealed that he had taken mineral oil four to five nights weekly for thirty years, and had used nose drops on many occasions. The chest roentgenograms revealed bilateral lower lobe disease with mottled densities in the medial halves, with a 2-cm. cavity in the right lower lobe, and a 2.5-cm. cavity in the left lingula. Sputum was microscopically positive for acid-fast bacilli. An intradermal skin test with 1:1,000 OT was positive, and coccidioidin and histoplasmin tests were negative.

Heavy growth of the acid-fast bacilli was obtained in ten days. The microorganisms grew rapidly on blood agar, Sabouraud medium, Hohn medium, nutrient broth, and Dubos liquid medium. Growth was only slightly slower at room temperature. The bacilli grew in medium containing 10 γ of streptomycin per ml. but not in concentration of 100 γ per ml. Growth was also obtained in 100 γ of PAS per ml., 10 γ of isoniazid and viomycin per ml., and 50 units of penicillin per ml. (partial growth in 100 units). Chloramphenicol, 10 γ per ml., did not delay growth of the organisms which were resistant also to amithiozone, thiazolsulfone, and cycloserine. They were moderately susceptible to chlortetracycline and oxytetracycline in concentrations of 10 γ per ml. The colonies were smooth, light cream in color, and dispersed readily in water. Exposure to light did not induce pigmentation. In old cultures, however, the growth became somewhat darker in color. The organisms did not produce disease in guinea pigs when given subcutaneously, intradermally, intraperitoneally, or into the pleural space, and only two of the pigs developed a feeble tuberculin sensitivity. Intratracheal injection, both with and without mineral oil, gave negative results. These acid-fast bacilli did not form cords.

Streptomycin and isoniazid were given to the patient because partial susceptibility was demonstrated to these two drugs, and they were discontinued after two weeks because of the development of hepatitis. A pleural effusion developed in September, 1955, and bacteriologic studies were entirely negative for fungi, acid-fast bacilli, and secondary organisms. Large doses of penicillin (25 million units per day) and sulfonamides were used, but had to be discontinued after ten days due to allergic manifestations. A Monaldi cavity drainage was performed on the right on February 14, 1956. Material from drainage showed the same type of acid-fast bacilli.

The patient's course in the ensuing year was marked by gradual reduction in the number of acid-fast bacilli in the sputum and Monaldi material. The cavity in the lingula of the left lung disappeared spontaneously. Mild diabetes mellitus was discovered in February, 1957, and evidence of renal disease was first noted in March, 1957. No acid-fast bacilli were found in the urine by microscopic or cultural methods. The patient died of renal failure on August 6, 1958. Permission for post-mortem examination could not be obtained.

In summary, this patient presents the problem of extensive pulmonary disease which may have been caused by atypical tubercle bacilli, or it may have been a lipoid pneumonitis with a superimposed acid-fast infection.

Case 4. G. R., a 33-year-old housewife and mother of three children, was admitted to the hospital on December 4, 1955, with an illness that dated to August, 1955, when a chest roentgenogram revealed abnormalities not noted in the last previous normal chest film of 1949. Family history revealed that two sisters and a brother had had tuberculosis, but the patient stated that there had

been no close contact during the time they were known to have active disease. An intradermal skin test with 1:100 OT was positive. A histoplasmin test with 1:100 dilution was positive. Complement-fixation tests for histoplasmosis and blastomycosis were negative. Three specimens of sputum at admission were positive for acid-fast bacilli, and the patient was started on isoniazid and PAS therapy. In January, 1956, the culture reports became available and it was noted that the colonies were not typical of tubercle bacilli; they were photochromogenic and did not form cords on subculture. Indirect drug-susceptibility tests revealed resistance to PAS and partial resistance to streptomycin, and to isoniazid. The strain of bacilli was incapable of producing progressive disease in the guinea pig. Chest roentgenograms showed infiltration in the upper posterior third on the right with suggestion of cavitation. Following recognition of the resistance to PAS, streptomycin was added to the drug regimen and resection of the diseased area was done on June 13, 1956. The pathologic report was "granulomatous inflammation of the lung with caseation and with encapsulation of smaller caseous lesions —the lesion in this case of atypical mycobacteriosis is indistinguishable from tuberculosis." The atypical tubercle bacilli were cultured from the surgical specimen, and the results of the susceptibility tests were similar to the preoperative findings. Following surgery, the sputum was consistently negative for acid-fast bacilli by all methods. The patient was discharged on September 22, 1956; drugs were continued until December 3, 1956. She continues well, and her three children showed no evidence of disease roentgenographically. Two sons, aged fourteen and five, were negative to intradermal tests with 0.1 ml. of 1:100 OT, and her daughter, aged thirteen, showed a minimal reaction to the same dosage.

In reviewing the findings, the only acid-fast bacilli found have been the atypical tubercle bacilli which were found in both the sputum and the granulomatous lesion removed at surgery.

Case 5. C. G., a 45-year-old female secretary, was admitted to the hospital on March 3, 1956.

A pre-employment roentgenogram in February, 1956, showed small infiltration in the upper right lung. Her family history was negative for tuberculosis. An intradermal skin test with 1:1,000 OT was positive. On four occasions sputum was positive for acid-fast bacilli by the concentration method, and on fourteen occasions atypical photochromogenic tubercle bacilli were cultured. In three of these cultures tubercle bacilli were also found. The atypical tubercle bacilli were resistant to streptomycin and to PAS, and were partially resistant to isoniazid. Cord-formation tests on the atypical bacilli were negative. The tubercle bacilli cultured were susceptible to streptomycin, PAS, and isoniazid; they were cord-formers and were pathogenic for guinea pigs. In May, 1956, drug treatment with streptomycin and isoniazid was started and was interrupted on several occasions because of allergic manifestations. Serial roentgenograms showed slight clearing of the disease. She left the hospital against advice on September 22, 1956. Laboratory studies at discharge showed that the sputum contained only the atypical tubercle bacilli. A recent communication stated that the patient was well and working.

This patient presents the problem of sputum containing both atypical and typical tubercle bacilli, with the former present in greater numbers and persisting after the tubercle bacilli could no longer be demonstrated.

Case 6. P. B., a 71-year-old porter, was transferred to this hospital on May 10, 1956. The patient's history of pulmonary disease dated back to about 1926, with onset of chronic cough; and in 1946, onset of frequent episodes of blood streaking. He was considered to have bronchiectasis with fibrosis and emphysema. Sputum was repeatedly negative for acid-fast bacilli. Onset of fatigue and loss of weight occured in 1952, and in 1954, cardiac disease with congestive failure was first noted. On April 15, 1956, he was again admitted to a hospital because of hemoptysis. Acid-fast bacilli were found in the sputum and drug treatment for tuberculosis was started. Subsequently, cultural study revealed that the bacilli did not have the usual characteristics of tubercle bacilli. An intradermal skin test with 1:1,000 OT was positive. Upon transfer to this hospital, the chest roentgenograms showed bilateral far advanced disease with infiltrations of a mottled hazy type, particularly in the lower two-thirds. The laboratory reported that the sputum cultures yielded acid-fast bacilli unlike tubercle bacilli. The colonies were soft and smooth and dispersed readily in water. The growth was nonphotochromogenic, non-cord-forming, and strongly catalase positive. Growth at 37°C. was slow, but the organisms were also capable of growth at room temperature. The strain was highly resistant to streptomycin and PAS, and to isoniazid on indirect testing. Subsequently the bacilli were resistant to 25 γ of viomycin per ml. and susceptible to 25 γ of cycloserine per ml. The strain did not produce disease in guinea pigs, but the pigs did develop sensitivity to tuberculin. The patient was continued on PAS and isoniazid, but the pulmonary disease continued to progress. On March 8, 1957, for the first time, two types of colonies were noted in the sputum cultures: one, an atypical tubercle bacillus and the other, typical *M. tuberculosis*. Subsequent cultures, totaling seven, showed only typical tubercle bacilli. These were resistant to isoniazid and PAS, but susceptible to streptomycin. The patient received PAS and isoniazid until February

6, 1957, and after that he refused all chemotherapy. His subsequent clinical course was characteristic of cardiac and renal failure. He died on December 5, 1958, and permission for post-mortem examination could not be obtained.

In summary, the patient had undiagnosed pulmonary disease for several years until atypical tubercle bacilli were found in April, 1956. Subsequently, typical tubercle bacilli were found and persisted until the patient's death, and none of the atypical type were noted in the last twenty-one months of his life.

Case 7. C. M., a 68-year-old telephone engineer, was admitted to this hospital on February 12, 1957. His first symptoms of pulmonary disease dated to 1911, when acid-fast bacilli were found in the sputum. Following treatment he returned to work and was well until September, 1955, when sputum became positive for tubercle bacilli. He was treated with streptomycin and PAS; a month later, isoniazid was added. In January, 1957, a laboratory elsewhere reported that the acid-fast bacilli were atypical. These bacilli did not produce disease in guinea pigs, hamsters, or chickens, and were nonphotochromogenic. On admission to this hospital, the chest roentgenograms taken in the previous sixteen months showed bilateral disease in the upper thirds, more extensive on the left, with cavity 1.5 by 4 cm. An intradermal skin test with 1:1,000 OT was positive. The acid-fast bacilli isolated from the sputum were classified as nonphotochromogenic atypical tubercle bacilli, although a light brown pigment did develop on exposure to light over an extended period of time. The colonies were smooth, and catalase activity was not as marked as with some other atypical strains. It was a non-cord-former, and grew as slowly at 37°C. as does *M. tuberculosis*. No growth was obtained in four to six weeks at room temperature. The bacilli were resistant to streptomycin and PAS, and susceptible to isoniazid and 25 γ of viomycin per ml.

Surgery was considered unwise, and it was advised that the patient continue on a rest and drug-therapy program. Since discharge from the hospital, the chest roentgenogram has not changed; sputum examinations are positive for atypical bacilli.

In reviewing this patient's problem, one can speculate as to whether the original disease was caused by these atypical tubercle bacilli, but inquiry has failed to produce any information to substantiate such a possibility.

Case 8. G. R., a 55-year-old welder, was admitted to this hospital on February 14, 1957. A chest roentgenogram taken in August, 1952, resulted in the diagnosis of active tuberculosis, and sputum culture revealed typical tubercle bacilli. No drug-susceptibility tests were done. He was admitted to a sanatorium in September, 1952, and was treated with streptomycin, 1 gm. three times weekly, and PAS, 12 gm. daily for one year. Sputum cultures continued positive and drug-susceptibility tests in July, 1954, showed resistance up to 300 γ of streptomycin per ml., and susceptibility to PAS and isoniazid. Isoniazid was added and the three drugs were continued until July, 1955, when the patient left the sanatorium against medical advice. At admission to this hospital, the chest roentgenograms showed bilateral disease with scattered nodular disease throughout the left lung and disease in the upper two-thirds of the right lung with a cavity 3.5 cm. by 7 cm. at the apex.

The sputum was found to be highly positive for acid-fast bacilli by the concentration method. Culture study revealed photochromogenic atypical tubercle bacilli. The growth rate was the same as that of *M. tuberculosis*, but the colonies were more readily dispersed in saline. The culture was a non-cord-former and was highly positive for catalase. Incubation at room temperature yielded growth after four to six weeks. A niacin test was negative. Indirect susceptibility to various drugs was tested and the strain was found to be completely resistant to streptomycin and PAS, and susceptible to 25 γ of isoniazid and viomycin per ml. The bacilli did not produce disease when inoculated into guinea pigs, but the animals developed a low sensitivity to OT. The patient has shown progression of his disease, and excisional surgery is planned for the right side.

The evidence in this case indicates that originally the patient had typical tubercle bacilli on the basis of the report of the sputum culture in August, 1952. Subsequently, only atypical tubercle bacilli were recovered, and during this latter period the patient has had progressive pulmonary disease.

Case 9. E. D., a 69-year-old man, was admitted to the hospital on February 21, 1957, with a history of having worked in coal mines for thirty-five years. A chest roentgenogram in November, 1956, revealed pulmonary disease. He was admitted to a hospital on January 24, 1957; his sputum was positive for acid-fast bacilli microscopically, and he was started on isoniazid and PAS therapy.

On admission to this hospital, an intradermal skin test with 1:1,000 OT was weakly positive. Chest roentgenograms revealed extensive disease typical of silicotuberculosis with fine scattered nodular disease and two small cavities, 1 by 2.5 cm., in the left upper lung field. The PAS and isoniazid were continued, and on March 13, 1957, 1 gm. of streptomycin daily was added. The patient died suddenly on March 19, 1957, following a myocardial infarction.

Post-mortem diagnosis revealed anthracosilicosis, emphysema, and fibrocaseous tuberculosis

of the lungs. The pathologist concluded: "Microscopically, all the tuberculous lesions showed poor encapsulation but as an unusual feature a very large number of giant cells in all lesions, with some irregular fibrosis throughout."

Subsequent to the patient's death, the reports of the admission sputum cultures became available. The cultures yielded atypical acid-fast bacilli. Growth on Hohn medium was nonphotochromogenic, smooth, and easily suspended. At room temperature a small inoculation in Dubos liquid medium produced noticeable growth in ten days. On Hohn medium at room temperature there was fine growth after six weeks. The strain was a non-cord-former and was highly active for catalase. The bacilli were short, evenly stained, and strongly acid fast. Drug-susceptibility tests indicated partial resistance to streptomycin and isoniazid and complete resistance to PAS.

Discussion

Since there are no distinctive clinical features by which the physician can recognize disease caused by or associated with "atypical tubercle bacilli," it is evident that the diagnosis is dependent upon the bacteriologist. It is therefore of greatest importance to carry out adequate cultural studies of the sputum before initiating drug therapy for tuberculosis.

The atypical bacilli described here differed from *M. tuberculosis* culturally as well as in ability to produce disease in the guinea pig. There is no evidence at this time that they are an end result of chemotherapy. Some of the patients had had no drug therapy prior to the recovery of these atypical mycobacteria, and had had no known contact with persons receiving drug therapy for tuberculosis. It is interesting and comforting to see that there is no evidence to date that the disease produced by atypical tubercle bacilli is contagious. This has important implications in the management of these patients with regard to the public health.

It will be noted that the patients are in the older age group, with only one under the age of forty years; this is in accord with other published reports. Such an age incidence lends support to the theory that lungs damaged by nontuberculous conditions such as emphysema or silicosis are more often the sites of disease associated with atypical tubercle bacilli; the assumption being that these organisms with low virulence are capable of producing disease in damaged lung tissue. Several but not all of the patients in the present series would fit into such a category. Classification of the patients' strains by Runyon's method (11) indicates that 5 are in the photochromogenic group, 3 in the nonphotochromogenic group, and one in the rapidly-growing group. In the photochromogenic group, clinical results were good in 4; the fifth patient (*Case 8*) has shown progressive disease. In the nonphotochromogenic group there were 2 deaths, apparently not due primarily to the pulmonary disease. In one of these (*Case 6*), atypical tubercle bacilli were found in the sputum during the last few months of life. The remaining patient in this group continues to have chronic active disease. In the patient with the rapidly-growing strain, death was due to renal disease rather than to progressive pulmonary disease.

Summary

The case reports are presented of 9 patients with "atypical tubercle bacilli" who have been admitted to the Ray Brook State Tuberculosis Hospital between 1952 and 1957. The bacteriologic properties of the organisms and the clinical courses of the patients are described.

Sumario

Afección Pulmonar Debida a Bacilos Tuberculosos Atípicos

Preséntanse las historias clínicas de 9 enfermos que tenían "bacilos tuberculosos atípicos" y fueron recibidos en el Hospital Estatal Ray Brook para Tuberculosis entre los años 1952 y 1957. Se describen las propiedades bacteriológicas de los microbios y la evolución clínica de los enfermos.

Resume

Maladie pulmonaire à bacilles tuberculeux atypiques

L'auteur rapporte 9 cas de maladie pulmonaire à bacilles tuberculeux atypiques, admis au Ray Brook Tuberculosis Hospital de 1952 à 1957. Il décrit le caractère bactériologique de ces organismes et l'évolution clinique des malades.

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Notes

GROWTH CHARACTERISTICS OF ACID-FAST MICROORGANISMS OTHER THAN TUBERCLE BACILLI IN HeLa CELLS

A search for methods helpful in differentiating so-called anonymous or atypical acid-fast bacilli from tubercle bacilli of low virulence and from saprophytes led to the use of HeLa cells as described by Shepard.[1-7] Strains of tubercle bacilli of varying virulence were first tested; but the method, which requires a battery of controls in order to evaluate slight differences in results, seemed too cumbersome for assessing virulence. With the atypical strains, however, growth within the HeLa cells occurred more rapidly, and in three days differences useful in distinguishing the varieties could be noted. About 100 strains were tested, and some of the more interesting ones were photographed.

The following cultures were studied: 7 strains of photochromogenic bacilli obtained from Dr. Ernest H. Runyon[8] of the Veterans Administration Hospital in Salt Lake City; 4 strains of photochromogenic bacilli isolated in this laboratory; and 6 received from a nearby laboratory; 8 strains from old, chronic cases of tuberculous disease received from Battey State Hospital, Rome, Georgia; 11 strains isolated in this laboratory from 4 similar cases; 12 scotochromogens isolated in this laboratory; one stock strain of avian tubercle bacilli; one transplant of Cuttino's[9] *Nocardia intracellularis*; one strain of *Nocardia asteroides* isolated in this laboratory; 3 strains of *Nocardia* spp.? from this laboratory; 48 assorted acid-fast microorganisms isolated in this laboratory which failed to conform exactly to any type. Many of the last-mentioned group were probably saprophytes.

The method used followed Shepard's technique fairly closely. The suspension contained 80,000 HeLa cells per milliliter in Hank's balanced salt solution plus 20 per cent human serum together with 100 γ of streptomycin, 50 units of penicillin, and 100 units of nystatin per milliliter, adjusted to pH 7.6–7.8. Coverslips, 9 by 22 mm., in Leighton tubes were covered with 1 ml. of the suspension and incubated in a horizontal position for two to three days (usually over the week-end). No antimicrobial drugs were used thereafter. The coverslips were then washed with balanced salt solution and covered with the same solution containing 40 per cent of a horse serum proved to be satisfactory, inoculated with 0.05 ml. of a dilute suspension of culture[1] and reincubated. The next day the coverslips were again washed and the medium was replaced with balanced salt solution containing 40 per cent human serum. This procedure was repeated every two days as long as the cultures were kept under incubation. Most of the cultures in this study were incubated for three days after inoculation.

At the end of the growth period the coverslip films were fixed in 2 ml. of 10 per cent neutral formalin for three hours. They were then dehydrated, stained with carbolfuchsin, decolorized, counterstained with Giemsa, dried, and mounted in gum dammar.

When the preparations were fixed and stained three days after addition of the culture, two groups could be distinguished by the appearance of the microorganisms; their arrangement differed markedly from the skeins formed by human and bovine tubercle bacilli (figure 1). The photochromogenic strains (Runyon's *Group I*),[10] as Shepard indicated, formed filaments with parallel sides and were markedly beaded (figures 2 and 3). The Battey strains (Runyon's *Group III*) were more irregular in shape with clubbed forms (figures 4 and 5). In most cases there was definite branching in portions of the slide where the microorganisms were thinly scattered (figure 6). On the whole, a larger proportion of the HeLa cells was infected with Battey strains and the clumps of microorganisms were larger; many cells appeared stuffed with the acid-fast bacilli. The growth of the photochromogenic strains was usually definitely less heavy.

The strains from 4 of the cases as well as avian tubercle bacilli and Cuttino's *Nocardia intracellularis* resembled the Battey group in growth and other characteristics.

[1] SHEPARD, C. C.: Proc. Soc. Exper. Biol. & Med., 1955, *90*, 392.

[2] SHEPARD, C. C.: J. Exper. Med., 1957, *105*, 39.

[3] SHEPARD, C. C.: J. Bact., 1957, *73*, 494.

[4] SHEPARD, C. C.: J. Bact., 1957, *73*, 722.

[5] SHEPARD, C. C.: Bact. Proc., 1957, *10*, 82 (abstract).

[6] SHEPARD, C. C.: J. Exper. Med., 1958, *107*, 237.

[7] SHEPARD, C. C.: Am. Rev. Tubere., 1958, 77, 968.

[8] TIMPE, A., AND RUNYON, E. H.: J. Lab. & Clin. Med., 1954, *44*, 202.

[9] CUTTINO, J. T., AND MCCABE, A. M.: Am. J. Path., 1949, *25*, 1.

[10] RUNYON, E. H.: M. Clin. North America, 1959 *43*, 273.

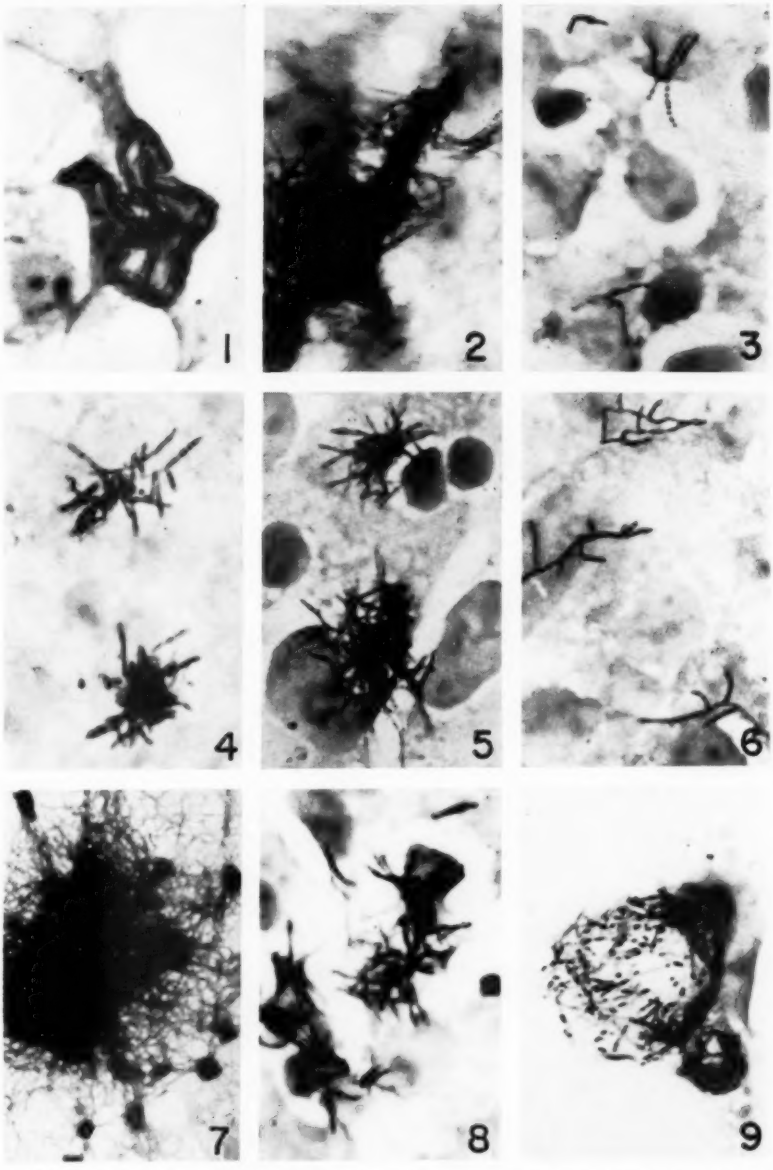

FIG. 1. Human tubercle bacilli in HeLa cell culture after five days' incubation showing skein form of the bacterial mass. (900×)

FIGS. 2 and 3. Photochromogenic bacilli isolated in the laboratory from 2 cases. The HeLa cell cultures for this and for all the succeeding plates were incubated for three days. (900×)

FIG. 4. Strain from a case in Battey State Hospital. (900×)

FIG. 5. "Battey" strain isolated in the laboratory from a fatal case of tuberculous disease. (900×)

FIG. 6. Another strain from Battey State Hospital. (900×)

FIG. 7. *Nocardia asteroides*. (900×)

FIG. 8. Scotochromogen. (900×)

FIG. 9. The "odd" strain. (900×)

The staining property of the *Nocardia asteroides*, the *Nocardia* spp.?, one of the strains from Battey State Hospital, and several unidentified strains isolated in this laboratory tended to be less acid fast than that of the others. The clumps in the inoculum of *Nocardia asteroides* could not be broken down sufficiently to permit the cells to engulf the microorganisms; the latter grew extracellularly in the medium (figure 7). The other microorganisms behaved similarly but had rather wider and shorter filaments and formed smaller clumps. The unidentified strains were considered to be saprophytes.

The photochromogenic strains, 17 of which have been studied thus far, could usually be easily differentiated microscopically from others by the beading, by the parallel regular sides, and by lack of branching. A strain was recently found to be photochromogenic that had been kept on medium since 1952, at which time it was saved from discard because it appeared to be unusual and possibly deserving of future study. The strain differed somewhat from the more recently isolated photochromogenic strains.

In the course of studying a group of cultures submitted for identification, one producing bright orange pigment both in the light and in the dark (scotochromogen—Runyon's *Group II*) was found to produce the same appearance as the photochromogens in HeLa cells. This observation led to the study of 11 similar strains, 2 of which showed this similarity to photochromogens. One of these 3 had been isolated from urine and one from gastric contents; the other one was received without history. These 3 are the only ones so far encountered with the same appearance as photochromogens.

Three additional scotochromogens were very irregular in shape and staining properties in the HeLa cells. The specimens from which 2 of these strains were isolated revealed short, thick, banded acid-fact bacilli in the original films; the third strain was isolated from a test specimen of unknown origin sent out by the Communicable Disease Center. All 6 of these orange strains tend to be intracellular in the HeLa cells.

The microorganisms in the remaining group of 6 orange cultures were solidly stained and nearly all were extracellular in HeLa cell preparations. Most of these were isolated under circumstances that indicate that they were contaminants. This group is illustrated in figure 8. Apparently, far from being a homogeneous group, the smooth-growing orange scotochromogens are members of two or possibly three groups and one of these is allied to the photochromogens. A number of nonchromogenic acid-fast strains, also considered to be probable contaminants, were very similar to these 6 orange strains in their appearance in HeLa cells. These considerations indicate that the presence or absence of pigment may have less significance than was formerly believed.

The strain shown in figure 9 differed entirely from all others tested. For this reason it has been designated the "odd" strain. It was isolated from a specimen submitted from a clinic patient, and it was impossible for the doctor to obtain additional specimens. The culture was moist, rather flat, and confluent. The strain was resistant to isoniazid, failed to induce lesions in a guinea pig, formed short, poor cords, was neutral red positive, and reacted strongly in the catalase test. The culture might possibly have been a mixture of resistant tubercle bacilli and the Battey type of bacilli or saprophytes; however, the original culture was sparse and appeared homogeneous. When the test in HeLa cells was repeated some months later, the bacilli were found to be more evenly stained and in smaller clumps, but the tendency persisted for the clumps to be rounded, with unstained spaces between the individual bacilli. Preparations incubated for seven days appeared the same as three-day ones. A guinea pig was inoculated with a suspension from the HeLa cell culture, but no lesions were found at the autopsy. A careful examination of slides from cultures with a similar type of growth revealed 6 others with this same trait; a stock dysgonic human strain was also similar. The histories of these cases, when available, indicated a chronic, relatively mild pulmonary infection not particularly resembling tuberculosis, and resistance to isoniazid. Three of the patients were in mental hospitals.

In summary: When studied in HeLa cells, Runyon's *Group I* strains seem to be uniform and so do the *Group III* strains. The latter show close relation to avian tubercle bacilli and Cuttino's *Nocardia intracellularis*. *Group II* strains, however, are far from uniform in their appearance in HeLa cells. A few closely resemble those of *Group I*, but the majority are not dissimilar to a group of unclassified nonchromogenic strains which are considered to be saprophytes.

The most interesting of all are the "odd" strains that seem to form vacuoles around the bacilli within the HeLa cells. Their similarity to dysgonic human cells in certain respects, their lack of many of the definitive characteristics of tubercle bacilli, and their association with a mild chronic form of pulmonary disease differentiate them from other groups.

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ISOLATION OF "ATYPICAL" MYCOBACTERIA FROM HEALTHY PERSONS

With increasing frequency in the last few years, acid-fast microorganisms—some of them well known, others recently recognized and popularly referred to as "atypical" mycobacteria—have been isolated from patients with clinical disease which often resembles tuberculosis.[1, 2, 3] The organisms frequently have been isolated in circumstances that incriminate them as causing the disease. In some instances, the causal relation is further supported by showing that the patients have strong reactions to PPD skin-test antigens made from the incriminated organism, and either no reaction or smaller (cross) reactions to the standard human type of tuberculin (PPD-S). Patients from whom typical tubercle bacilli are isolated, on the other hand, have strong reactions to PPD-S, and either no reaction or smaller reactions to the other antigens.[4]

Current knowledge suggests that clinical disease results much less often from infection with the atypical mycobacteria than from infection with typical organisms. In the states of Georgia and Florida, for instance, more than two-thirds of the young men recruited for Navy service were classified as reactors to the PPD antigen made from the "Battey" Mycobacterium,[5] while only about 6 per cent of them were considered to be reactors to PPD-S.[4] In spite of this marked disparity in the prevalence of infection, recognized cases of atypical mycobacterial disease comprise only a very small proportion of the total cases admitted to the tuberculosis hospitals in these states. It would therefore seem likely that most reactors to the PPD made from the Battey Mycobacterium which, in these states, includes most of the adults, have been sensitized as the result of a benign asymptomatic infection. If this concept is correct, it would be expected that atypical mycobacteria might be isolated from apparently healthy persons. The present study was made to investigate that possibility.

Tuberculosis studies carried out by the Muscogee County (Georgia) Health Department during the last ten years have included occasional surveys—skin tests and roentgenograms—of an isolated, self-sustaining community of about 375 persons, Negro workers and their families, living on the Bradley Plantations about 30 miles south of Columbus, Georgia. The plantations are a commercial farming operation (cotton, corn, oats, peanuts) subdivided into five "farms," each managed by a resident overseer. The workers and their families on each farm live in homes grouped around their vegetable gardens and near the company store from which they obtain staple foods, tobacco, and other necessities.

The main result of the surveys conducted on the plantations has been the finding of very little evidence of tuberculous infection and disease in the population. On the other hand, a high prevalence of nonspecific tuberculin sensitivity has been found. For example, in a survey in 1956, tests were given simultaneously with PPD-S and three other PPD antigens: avian tuberculin, Weybridge; PPD-C, prepared from the acid-fast organism isolated by Cuttino and McCabe;[6] and PPD-M, from a chromogenic Mycobacterium isolated in India.[7] The Battey-PPD was not available at that time, although subsequent studies have shown it to give results very similar to those obtained with the three other antigens.[8] Reactions of 10 mm. or more to PPD-S were found in only 2.6 per cent of the persons less than twenty years of age in 1956, and in every instance the reaction was smaller to PPD-S than it was to the other antigens. In the adult group, 30 per cent had a reaction of 10 mm. or more to PPD-S, but the reactions were larger to the other antigens than to PPD-S in one-third of them.

For a number of reasons, not the least important of which was almost complete participation of the population in the earlier surveys, this community was selected as a suitable one for trying to isolate atypical myobacteria from apparently healthy persons. Arrangements were made by Dr. Harold Keltz in January, 1958, to collect sputum specimens from as many as possible of the persons more than fourteen years of age. Participation was again remarkably complete: nearly all of the adult residents (a total of 122 persons) cooperated to the best of their ability. Each person, individually,

[1] Pollak, A., and Buhler, V. B.: Am. J. Path., 1951, *27*, 713.

[2] Crow, H. E., *et al.*: Am. Rev. Tubere., 1957, *75*, 199.

[3] Hardy, A. V., *et al.*: Am. J. Pub. Health, 1958, *48*, 754.

[4] Edwards, L. B., and Palmer, C. E.: Am. J. Hyg., 1958, *68*, 213.

[5] So called because this organism was first reported from the Battey State Hospital, Rome, Georgia.[3]

[6] Cuttino, J., and McCabe, A.: Am. J. Path., 1949, *25*, 1.

[7] Edwards, L. B., and Krohn, E. F.: Am. J. Hyg., 1957, *66*, 253.

[8] Edwards, L. B., *et al.*: Acta tubere. scandinav., 1959 (Supplement 47, p. 77).

was asked to cough "from the bottom of the chest"; he was shown how this was done, and was encouraged to try repeatedly. As long as five or ten minutes were sometimes spent with one person in an attempt to make the cough productive. A sterile glass sputum container was used to collect whatever the person could produce. Some of the specimens appeared to be sputum, but many of them were just saliva.

All of the specimens, regardless of physical appearance, were labeled and taken to the laboratory of the Muscogee County Health Department where the bacteriologic examinations were made.[9] The specimens were digested with sodium hydroxide, centrifuged, and neutralized, and the sediment from each was planted on one slant of modified Löwenstein-Jensen medium. The tubes were placed in the incubator and were examined for growth at weekly intervals. Primary isolations were made from specimens for 29 persons. In most instances only a single colony appeared in the tubes; in one instance, two different types of colonies were found in the same tube. Specimens for 92 of the 122 persons showed no growth whatever; one other had a colony thought to be acid-fast bacilli, but it became overgrown with "aerial mycelium" and the colony was lost.

Subcultures of the 30 strains were sent to Dr. Ruth Gordon at Rutgers University, who classified 14 of them as Actinomycetaceae, 3 as *Mycobacterium fortuitum*, and 13 simply as mycobacteria. Of the 14 strains identified as Actinomycetaceae, one was classified as *Nocardia asteroides*, 10 as similar to *Nocardia madurae*, 2 as similar to *Streptomyces rimosus*, and one as similar to *Str. sulfureus*.

The 16 mycobacterial strains were forwarded to Dr. Ernest Runyon of the Veterans Administration Hospital, Salt Lake City, for further identification according to his four groups.[10] He found no typical tubercle bacilli and no *Group I* (photochromogenic) organisms. Three of the cultures were *Group II* (scotochromogens); 7 were *Group III* (nonphotochromogens, Battey type); 5 were *Group IV* (rapid growers); and one was a mixed culture of both *Group II* and *Group III* organisms. Among the 5 classified as *Group IV* were the 3 classified by Dr. Gordon as *M. fortuitum*.

As shown in table 1, positive cultures were obtained as frequently from the younger as from the older persons, although it may be of some significance that, in all but one instance among the younger group, mycobacteria were recovered from specimens from men. Another possibly significant finding was that organisms were isolated almost as often from specimens that looked like saliva as from those that appeared to contain some sputum. Finally, positive cultures were more frequent in persons from two of the five farms: about 40 per cent of the specimens collected from residents of those two farms were positive, compared with about 15 per cent on two of the other farms, and none at all on the fifth farm.

TABLE 1

RESULTS OF BACTERIOLOGIC EXAMINATIONS OF SPUTUM OR SALIVA SPECIMENS FROM 122 HEALTHY ADULTS

Bacteriologic Results	Study Population				
	15–39 Years		40 Years and Older		Total
	Male	Female	Male	Female	
Number of specimens	32	34	26	30	122
No growth	21	28	18	25	92
Growth	11	6	8*	5	30*
Mycobacteria					
Group I	—	—	—	—	0
Group II	2†	—	1	1	4
Group III	4†	1	1	2‡	8
Group IV	3	—	—	2	5
Actinomycetaceae	3	5	5	1‡	14

* Includes one culture which was lost.

† One culture had both *Group II* and *Group III* organisms.

‡ One culture had both *Group III* and *N. madurae* organisms.

When the specimens were being collected, it was noted that both snuff and chewing tobacco were in common use on the plantations. Later, when such a high proportion of the cultures proved to be positive, samples of snuff and tobacco were obtained from the company store for bacteriologic culture. No growth was reported from any of the samples.

Readings of the chest roentgenograms taken in the 1956 survey of the Bradley Plantations were recently reviewed in the light of the present findings. Films were available for 106 of the 122 persons from whom sputum specimens had been obtained. Only 3 had any evidence whatever of possible pulmonary pathology; and those 3 were all in the group with negative bacteriologic cultures. The films for the 29 persons with positive cultures were all read as normal; and new films obtained in 1958 for all but 2 of the 29 were again read as normal.

* * *

The isolation of nonphotochromogenic organisms of the Battey type from the sputum or

[9] Miss Martha Jane Spence performed the laboratory examinations.

[10] RUNYON, E. H.: M. Clin. North America, 1959, *43*, 273.

saliva of 8 of 122 healthy persons living on a plantation in southern Georgia is of particular interest because, thus far, most of the clinical cases of disease attributed to the Battey organism have been from Georgia[2] and the neighboring state of Florida.[3] While the present findings do not, by themselves, prove infection of healthy people by the organism, they certainly suggest that the organism must be very prevalent and readily accessible to large numbers of persons.

The same may apply to the finding of the other mycobacteria and the Actinomycetaceae. These organisms, when encountered in routine bacteriologic work, often may be regarded as contaminants and discarded; yet, recently, both the scotochromogens and the rapid growers have been incriminated as occasionally causing disease in man.

The finding that half of the positive specimens appeared to be only saliva and not sputum might be a clue to the epidemiology of these infections: the organisms may be transmitted by food or water. In an unpublished study, a single oral inoculation of a suspension of the Battey organisms generally sensitized guinea pigs to the Battey PPD. It therefore seems possible that the gastrointestinal tract may be a frequent portal of entry for natural infection of human populations with some of the atypical mycobacteria.

The present study is obviously a small one, but the results are sufficiently suggestive to warrant the study of larger and more representative population groups. If organisms were isolated only from reactors to the mycobacterial antigens, and not from nonreactors, the evidence would be strengthened that some of the atypical mycobacteria cause a benign infection in vast numbers of persons, and produce the skin sensitivity which is so highly prevalent to the Battey and related antigens (as well as cross-sensitivity to PPD-S). In order to have sufficient numbers of nonreactors, however, such studies could most profitably be done in areas where the prevalence of sensitivity to the atypical mycobacterial antigens is considerably lower than it is in the southeastern part of the country.

In summary: Atypical mycobacteria were isolated from the sputum or saliva of a surprisingly high proportion of the healthy adult population of a small rural community in southwestern Georgia.

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THE RELATIONSHIP OF SEX TO THE SUSCEPTIBILITY OF NORMAL AND IMMUNIZED MICE TO TUBERCULOSIS[1]

The relationship of sex to the susceptibility of animals to tuberculosis has long been of interest to investigators in the field. It is generally agreed that women are more susceptible during their child-bearing years, and particularly during the gestation period. Experimentally, it has been demonstrated that the susceptibility of animals to tuberculosis can be increased by the administration of estrogenic hormone.[2-5]

In 1950,[6] while defining the conditions for a standardized tuberculous infection in mice, experiments were conducted to determine the relative susceptibility of 20 gm. male and female "Strong A" mice to an infecting dose of 1.0 gm. of the H37Rv strain of *Mycobacterium tuberculosis*. Although the median survival time of the male mice was slightly longer than that of the females, the difference was not statistically significant. It was concluded from these experiments that there was no real difference in susceptibility of male and female "Strong A" mice to infection with the H37Rv strain of *M. tuberculosis*. Similar conclusions had been reached by Donovick and associates, using CF_1 mice.[7] Furthermore, there is no striking indication from the extensive data of Swedberg[5] that the females from an inbred strain of white mice were any more susceptible to infection with a bovine strain of *M. tuberculosis* than were the male mice of the same strain.

In 1957, however, Hoyt and co-workers[8] reported that female mice of the "Olitsky" strain were more susceptible to infection with the H37Rv strain of *M. tuberculosis* than were the males. The differences noted by these writers between the mean survival times of the male and female mice were small, but were statistically significant when large numbers of animals were employed. The increased resistance of male mice to tuberculous infection was appreciably more marked when the infecting dose of the H37Rv strain was administered following vaccination with BCG. Apparently the male mice responded more effectively to immunization than did female mice.

The report of Hoyt and associates stimulated the present writers to examine the data that had been collected, using both immunized and non-immunized animals, since the first publication from this laboratory on the susceptibility of female and male mice to tuberculous infection. In addition, beginning early in 1958, experiments were initiated in which male and female mice were used purposely in the same experiments. Prior to this time, for the most part, experiments were conducted with mice of only one sex and, because of their greater docility, female mice were employed more frequently than males.

The standardized mouse infection employed in these experiments has been described elsewhere.[6] It will suffice to point out that all mice were infected intravenously with a 1.0-mg. dose of the H37Rv strain of *M. tuberculosis*. All vaccinations were performed by intraperitoneal administration of the immunizing agent, and all mice were challenged 28 days after immunization. The effectiveness of the immunization procedure was determined by comparing the number of immunized mice which survived 30 days or longer with the number of control mice which survived for the same period. Since normal distributions of deaths occurred among the control mice, comparisons also were made on the basis of the mean and median survival times. The validity of these evaluation procedures has been demonstrated and has been reported in detail in another communication.[9]

The data obtained from 1952 to 1959 on the relative susceptibility of both normal and vaccinated mice to infection with *M. tuberculosis* are summarized in table 1. These data have been divided into two groups: those mice infected from 1953 through 1959, and those infected only during 1958 and 1959. The data from many experiments have been pooled and grouped according to the immunizing agent employed.

Over the 1952–1959 period, 196 male mice were

[1] Supported in part by research grant (E-1636) from the National Institutes of Health, Public Health Service, and a grant from the Tuberculosis Institute of Chicago and Cook County.

[2] LURIE, M. B., *et al.*: Am. Rev. Tuberc., 1956, *59*, 168.

[3] LURIE, M. B., *et al.*: Am. Rev. Tuberc., 1949, *59*, 186.

[4] LURIE M. B., *et al.*: Am. Rev. Tuberc., 1949, *59*, 198.

[5] SWEDBERG, B.: Acta med. scandinav., 1951, 139 (Supplement 254).

[6] YOUMANS, G. P., AND YOUMANS, A. S.: Am. Rev. Tuberc., 1951, *64*, 541.

[7] DONOVICK, R., *et al.*: Statistics for Medical Students, The Blakiston Co., New York, 1951.

[8] HOYT, A., *et al.*: Am. Rev. Tuberc., 1957, *75*, 618.

[9] YOUMANS, G. P., AND YOUMANS, A. S.: J. Immunol., 1957, *78*, 318.

TABLE 1

SUSCEPTIBILITY OF NORMAL AND IMMUNIZED MALE AND FEMALE MICE TO INFECTION WITH *M. TUBERCULOSIS*

Experimental Mouse Groups	Sex	Number of Mice	Number of S-30* Mice	Percentage of S-30* Mice	Survival Percentage Difference Male-Female	P
BCG-vaccinated, 1952–1959	Male	196	121	61.7	6.4	<0.20–>0.10
	Female	217	120	55.3		
H37Ra-vaccinated, 1953–1959	Male	404	235	58.0	12.2	<0.001
	Female	880	424	45.9		
Controls 1953–1959	Male	479	31	6.4	2.9	0.01
	Female	842	29	3.5		
H37Ra-vaccinated, 1958–1959	Male	261	141	54.0	11.9	0.01
	Female	202	85	42.1		
Controls, 1958–1959	Male	262	13	4.96	2.05	<0.30 >0.25
	Female	206	6	2.91		

* Survived longer than 30 days.

vaccinated with BCG and 217 female mice were vaccinated with the same agent. The percentage of male mice that survived longer than 30 days was 61.7; female, 55.3. Statistically, as determined by the chi-square test, there was no significant difference between the response of these male and female mice to immunization with BCG. However, these data do not provide an adequate test of the relative susceptibility of male and female mice following immunization to infection with *M. tuberculosis* because not only were 4 different BCG strains employed but the BCG cells in some cases were lyophilized, and in some cases were heat-killed. Since the number of male and female mice was not uniformly distributed in each of the experiments utilizing these different preparations, the data cannot be used as a valid measure of the effect of sex on response to immunization.

The pooled data from all of the experiments conducted using living H37Ra cells as an immunizing agent are much more suitable for analysis. In all of these experiments, although conducted over a period of years, all mice were immunized intraperitoneally with 1.0 mg. of the attenuated H37Ra strain. The percentage of the H37Ra immunized male mice which survived longer than 30 days was 58.1, whereas only 45.9 per cent of the female mice survived longer than 30 days. Statistically, this difference is highly significant since $P = 0.001$.

The results with the control mice from these experiments also have been pooled and 31 of 479 male mice survived longer than 30 days following infection, whereas only 29 of 842 female mice survived longer than 30 days. The per cent of control male and female mice which survived 30 days was 6.4 and 3.5, respectively. Statistically, the male mice appeared to be more resistant to the tuberculous infection. The mean survival time of the male mice was 16.75 days and of the female mice 15.05 days. Statistically, these means also differed significantly, $P = < 0.001$. The median survival time of the males was 16.0 days (15.8–16.2); of the females, 14.5 (14.3–14.7). Statistically the difference, at the 95 per cent level was of questionable significance when analyzed by the method of Litchfield.[10]

The preceding data indicate strongly that "Strong A" female mice are more susceptible to infection. However, this conclusion is derived from data obtained over a period of several years and from experiments in which, for the most part, only male or female mice were employed. The highly standardized nature of the experimental tuberculous infection employed reduces error from these factors, but, in order to completely eliminate the possibility of such an error, in 1958 and continuing into 1959, 14 different experiments were set up in which female and male mice were

[10] LITCHFIELD, J. T.: J. Pharmacol. & Exper. Therap., 1949, *97*, 339.

used in each experiment. The pooled data from these experiments have been analyzed separately and are shown in table 1. Following immunization, 54.0 per cent of the male mice survived longer than 30 days, whereas only 42.1 per cent of the female mice survived longer than 30 days, a highly significant difference. Of the control mice, 4.96 per cent of the male mice survived longer than 30 days, whereas only 2.0 per cent of the females survived longer than 30 days. This difference on analysis by the chi-square test did not prove to be statistically significant.

Since so few of either the control male or female mice survived longer than 30 days, the mean survival time of the male mice was calculated and found to be 17.0 days. The mean survival time of the female mice was 16.0 days. When analyzed statistically, this difference was not significant since P = >0.5. The median survival time of the males was 16.0 days; of the females, 15.0 days. Again, the difference was not found to be statistically significant.

It is clear that the results of the analysis of our accumulated data with the "Strong A" strain confirm the findings of Hoyt and his colleagues,[8] using the "Olitsky" strain, that male mice respond more effectively to immunization against tuberculous infection than do female mice. Although the difference in response to immunization between male and female mice is not extreme, it is large enough to be taken into consideration in immunization experiments in which differences in immunizing potency of vaccines are being sought.

It also appears that nonimmunized female mice may be more susceptible to tuberculous infection. However, this difference is slight and, even in experiments employing large numbers of animals, may not be apparent. In this connection it is of interest that Hoyt and co-workers[8] noted a highly significant difference (P = <0.001) between the susceptibility of their control male and female animals when they pooled the data from all of their BCG vaccination experiments. However, in a summary of five experiments which involved 136 male and 124 female control animals, respectively, these writers found no statistically significant difference between the susceptibility of the male and female animals.

In summary: The analysis of data collected over a period of several years showed that male "Strong A" mice responded to immunization with BCG vaccine and viable H37Ra cells to a significantly greater degree than did female mice of the same strain. The number of male immunized mice which survived longer than 30 days was about 12 per cent greater than the percentage of female mice which survived for the same period.

When nonimmunized mice of the "Strong A" strain were infected with virulent tubercle bacilli, the number of male mice which survived longer than 30 days was significantly larger than the number of female mice which survived the same period. Moreover, when large numbers of animals were employed, the mean survival time of the male mice, which died in less than 30 days, was significantly longer than the mean survival time of the female mice, which died in less than 30 days.

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THE DIFFERENCE IN RESPONSE OF FOUR STRAINS OF MICE TO IMMUNIZATION AGAINST TUBERCULOUS INFECTION[1]

In recent years the mouse has become an increasingly popular animal for the study of host-parasite relationships in tuberculosis. In particular, many investigators interested in defining the nature of the immunizing substance, or substances, in tubercle bacilli have used the mouse to measure the immune response. No attention, however, has been paid to the influence of the mouse strain on the immunization response. Most investigators apparently either procure and use a mouse strain which has been employed successfully elsewhere or use the most readily accessible mouse supply.

When studies in experimental murine tuberculosis were initiated in this laboratory in 1944, the availability of "Strong A" mice from a colony maintained in this institution by Dr. Frances D. Gunn dictated the choice of mouse strain. This strain proved to be very satisfactory for studies on the experimental chemotherapy of tuberculosis, and, when interest expanded into the realm of the immunology of tuberculosis, the same strain was continued in use. A highly standardized tuberculous infection in this strain of mouse was developed,[2] and studies have been reported on the nature of the pathologic process.[3, 4] The evaluation of the response of this strain of mice to immunization with a variety of immunizing agents has also been the subject of considerable investigation, and an analysis has been made of the nature of the immune response, both in terms of survival time and of the multiplication of tubercle bacilli in the lungs, spleen, and liver.[2, 5-12]

During the course of these investigations, there have been three occasions when insufficient animals were available from the breeding program to meet the experimental requirements. Therefore, other strains of mice were purchased and used. Experience with one of these, the Rockland "all-purpose strain," has already been reported[5] and it was pointed out that this strain did not immunize as well as did the Strong A strain, although the difference between the two strains was not marked.

Since that time, there has been an opportunity to test two other strains of mice. In view of the great differences encountered in the capacity of these strains to develop immunity to viable antituberculous vaccines, it was thought to be advisable to present the results of the studies with all four strains of mice.

The immunization and challenge procedures and the method of evaluating the immune response in mice have been presented previously.[5] It is only necessary here to point out that all immunizing injections were given by the intraperitoneal route and that all mice were challenged intravenously with 1.0 mg. of the H37Rv strain of *M. tuberculosis* 28 days following immunization.

Furthermore, in all cases, prior to utilization of any one of the strains of mice for immunization purposes, experiments were conducted to determine their susceptibility to the standard intravenous infecting dose of 1.0 mg. of the H37Rv strain.[2] In no case was there evidence of any significant difference in susceptibility to infection of these 18–22 gm. mice. The median survival times of all 4 strains ranged between 13 and 15 days.

The results obtained following immunization of the 4 strains of mice are summarized in table 1. The Rockland "all-purpose" strain was obtained from a local breeder. Mice of the Rockland "specific pathogen-free" strain were obtained from Rockland Farms. The CF_1 mice were purchased from Carworth Farm.

The data given in table 1, in each case, have been pooled from the results of several experiments run at different times. Analysis of the pooled data provides a more sensitive measure of differences between strains of mice, although similar differences could be demonstrated in in-

[1] Supported in part by research grant (E-1636) from the National Institutes of Health, Public Health Service, and a grant from the Tuberculosis Institute of Chicago and Cook County.

[2] YOUMANS, G. P., AND YOUMANS, A. S.: Am. Rev. Tuberc., 1951, *64*, 541.

[3] RALEIGH, G. W., AND YOUMANS, G. P.: J. Infect. Dis., 1948, *82*, 197.

[4] RALEIGH, G. W., AND YOUMANS, G. P.: J. Infect. Dis., 1948, *82*, 205.

[5] YOUMANS, G. P., AND YOUMANS, A. S.: J. Immunol., 1957, *78*, 318.

[6] YOUMANS, G. P., *et al.*: J. Bact., 1955, *70*, 557.

[7] YOUMANS, A. S., *et al.*: Proc. Soc. Exper. Biol. & Med., 1957, *96*, 762.

[8] YOUMANS, A. S., AND YOUMANS, G. P.: Proc. Soc. Exper. Biol. & Med., 1955, *90*, 238.

[9] SEVER, J., AND YOUMANS, G. P.: Am. Rev. Tuberc., 1957, *75*, 280.

[10] SEVER, J., AND YOUMANS, G. P.: Am. Rev. Tuberc., 1957, *76*, 616.

[11] SEVER, J., AND YOUMANS, G. P.: J. Infect. Dis., 1957, *101*, 193.

[12] YOUMANS, G. P., AND YOUMANS, A. S.: Proc. Soc. Exper. Biol. & Med., 1956, *92*, 566.

TABLE 1

RESPONSE OF FOUR STRAINS OF MICE TO IMMUNIZATION AGAINST AN EXPERIMENTAL TUBERCULOUS INFECTION

Mouse Strain	Sex	Immunizing Agent	Infecting Dose of H37Rv in Milligrams	Number of Mice	Number of S-30* Mice	Percentage of S-30* Mice
"Strong A"	M	BCG	1.0	196	121	61.7
	F	BCG	1.0	217	120	55.3
	M, F	BCG	2.0	39	6	15.3
"Strong A"	M	H37Ra	1.0	404	235	58.1
	F	H37Ra	1.0	880	424	45.9
	M, F	H37Ra	2.0		35	11.0
"Strong A"	M, F	"Red fraction"	1.0	151	71	47.01
Rockland "All-purpose"	F	BCG-4	1.0	134	46	35.7
Rockland "Specific pathogen-free"	F	H37Ra	1.0	150	30	20.0
		H37Ra	2.0	20	0	0.0
"CF_1"	M	H37Ra	1.0	223	199	89.2
		H37Ra	2.0	19	11	58.0
"CF_1"	M	BCG	1.0	72	71	98.6
"CF_1"	M	"Red fraction"	1.0	147	60	40.8

* S-30 = Survived longer than 30 days.
M = Male
F = Female

BCG vaccine supplied through the courtesy of Dr. Sol R. Rosenthal, Tice Clinic, University of Illinois, Chicago, Illinois.

dividual experiments. The highly standardized nature of the particular tuberculous infection in mice employed in the studies makes a comparison of the results obtained with these strains valid even though the tests were conducted at different times.[12] However, experiments with the Strong A and CF_1 mice were in several instances conducted within one to two weeks of each other.

For each immunization experiment, control nonimmunized mice were distributed in the cages at the same time when other similar mice were immunized. These nonimmunized control mice were then challenged 28 days later with the same infecting dose used for the immunized animal. Homologous controls are essential in such experiments because the mice increase in age and weight over the immunization period and are, therefore, somewhat more resistant to infection.[5] Again, however, there was little evidence to indicate that the susceptibility of the nonimmunized control mice of these 4 strains to experimental tuberculous infection varied significantly. The median survival times of these older and heavier control mice were: Strong A mice, 19 days; Rockland all-purpose mice, 16 days; Rockland specific pathogen-free mice, 15.0 days; CF_1 mice, 18.0 days.

The effectiveness of vaccination has been indicated in table 1 by recording the number of mice which survived longer than 30 days. In a previous publication it was pointed out that Strong A mice immunized against tuberculosis can be divided on the basis of their survival times into two populations.[5] The first consists of those mice which die of an acute progressive infection within the first 30 days, the remainder consist of those mice which live for prolonged periods and suffer from a chronic slowly progressive tuberculous infection. Ninety to 95 per cent of the control animals usually will die of the acute progressive infection in less than 30 days. The effectiveness of vaccination can be measured most satisfactorily by calculating the percentage of mice in which the infection develops as a chronic infection rather than as an acute rapidly fatal infection.

The data on Strong A mice in table 1 vaccinated with BCG are the same as those given in the previous article;[12] other similar data can be found in footnote[5]. The results obtained with the Rockland all-purpose strain of mice have been taken from footnote[5]. It is apparent that, as has been previously reported, the Rockland all-purpose strain of mice immunized with BCG-4 showed less response to immunization than did the Strong A mice immunized also with BCG-4. Furthermore, the Rockland all-purpose strain of mice responded more poorly to immunization with BCG-4 than did Strong A mice immunized with H37Ra. Nevertheless, it is clear from these data and from data previously given,[5] that BCG-4 is a slightly better immunizing agent in Strong A mice than is H37Ra. The fact that the Rockland all-purpose mice were all females does not alter the validity of these conclusions since Strong A female mice immunized with BCG-4 consistently showed that more than 50 per cent of the animals survived 30 days.

[12] YOUMANS, G. P., AND YOUMANS, A. S.: Am. Rev. Resp. Dis., 1959, *80*, 750.

The Rockland specific pathogen-free mice also were all females, and only three experiments utilizing 150 mice were performed, since it was evident early in the experiments that immunization against tubercle bacilli was less effective in this strain of mouse. Only 20 per cent of 150 mice immunized with 1 mg. of H37Ra survived longer than 30 days; a very significantly lower response to immunization than that obtained with either the Strong A or the Rockland all-purpose strain.

The most striking and significant findings, however, were obtained with mice of the CF_1 strain. These were all males, and comparisons can be made with Strong A male mice vaccinated with H37Ra and BCG. Approximately 90 per cent of 223 CF_1 male mice in six separate experiments when immunized with 1 mg. of H37Ra survived over 30 days, a striking difference from the response seen in the other three strains of mice, even though equivalent data in Rockland all-purpose and pathogen-free male mice are not available. Even more dramatic is the response of the CF_1 mice to immunization with the BCG-4 strain. Only one mouse of 72 in two experiments failed to survive longer than 30 days.

Other data are available in table 1 which emphasize the superiority of the immunizing capacity of the CF_1 male mice over mice of the other strains. Immunized mice of three strains, the Strong A, the specific pathogen-free, and the CF_1 mice were challenged with 2 mg. of the virulent H37Rv strain instead of the usual 1.0 mg. challenge dose. This challenge dose of H37Rv in Strong A mice, as indicated in the table and from data previously published,[5] almost completely eliminates any indication of acquired immunity. However, 58 per cent of the immunized CF_1 mice challenged with 2.0 mg. survived longer than 30 days. In other words, the percentage of mice which survived more than 30 days after they had been injected with 2.0 mg. of the virulent H37Rv cultures was as large with CF_1 mice as the percentage of Strong A mice which survived more than 30 days following challenge with only 1.0 mg. of H37Rv.

Strong A and CF_1 strains also were immunized with "red fraction." This is a highly immunogenic enzymatically active particulate material obtained by ultracentrifugation from extracts prepared from mechanically ruptured living H37Ra cells.[6, 7] It has been the writers' experience in numerous experiments that red fraction in Strong A mice almost equals in immunizing power the viable intact cells from which it is prepared.[6, 7] This is confirmed by the data in table 1. It would be expected, in view of the much greater immune response of CF_1 mice to intact bacilli, that they would show also a much greater immune response to red fraction. However, in five experiments involving 147 mice, the immune response to red fraction in CF_1 mice was actually a little less than that found in Strong A mice.

* * *

Two major points of interest emerge from the findings reported in this paper. First, it is clear that there was little or no difference in susceptibility to infection with the 1.0 mg. dose of H37Rv between nonimmunized mice of the four strains tested. This finding is in agreement with the findings of Donovick and associates[14] who noted little difference in susceptibility to infection with the Ravenel strain of *Mycobacterium* var. *bovis* between seven strains of mice.

The present findings and those of Donovick and associates are, however, in conflict with those of Pierce and co-workers,[15] who tested the susceptibility to infection of twenty-two strains of mice using *M. tuberculosis* as the infecting agent. They found significant differences in susceptibility to tuberculous infection between many of the mouse strains, including the Strong A strain and many of the mouse strains tested by Donovick and associates. Both Donovick and associates and the present writers used large infecting doses of the respective mycobacteria which produced an acute, progressive, and rapidly fatal infection in all mice. Pierce and co-workers, on the other hand, used appreciably smaller infecting doses.

In recent years, data have become available which show clearly what happens in Strong A mice following infection with virulent human tubercle bacilli of the H37Rv strain. When Strong A mice are intravenously infected with large doses (1.0 mg.), the tubercle bacilli, after a short lag, begin to multiply and increase in number exponentially at about one-half the maximal rate which can be achieved *in vitro*.[10] Multiplication continues at this rate until death, which occurs when a certain number of tubercle bacilli have accumulated in lungs.[10] Meynell and Meynell[16] have provided data which show that a similar sequence of events occurs in experimental infection with many other pathogenic microorganisms. When Strong A mice are infected with smaller infecting doses (0.01 to 0.25 mg.), multiplication of the infecting tubercle bacilli proceeds initially at the same rate as that which occurs following in-

[14] Donovick, R., *et al.*: Am. Rev. Tuberc., 1949, *60*, 109.

[15] Pierce, C., *et al.*: J. Exper. Med., 1947, *86*, 159.

[16] Meynell, G. G., and Meynell, E. W.: J. Hyg., 1958, *56*, 323.

jection of the large inocula. However, the mycobacteria in the lungs frequently do not reach the lethal number by the time the forces of acquired immunity, which are stimulated by the infecting dose itself, become active. Multiplication of the tubercle bacilli then will abruptly cease and the animal will develop a chronic very slowly progressive infection which will bring about death only after a period of weeks or months. It follows, from this, that innate, or natural, resistance to tuberculosis can only be measured in mice by inoculating infecting doses of mycobacteria large enough so that death will supervene before the forces of acquired immunity can be brought into play. It also follows, when small infecting doses are used, that the average survival time of the mice may be a measure of the capacity of the animals to develop acquired resistance, rather than a measure of natural resistance. It seems probable, in view of the smaller infecting doses employed by Pierce and Dubos and by Middlebrook, that these writers actually were measuring differences in the capacity of the strains of mice to develop acquired resistance to tuberculosis, rather than differences in natural resistance.

Second, strains of mice may show profound differences in their capacity to develop resistance against tuberculous infection following vaccination with viable attenuated strains of mycobacteria. It is equally evident, however, that two strains of mice which vary markedly in their response to immunization with viable attenuated mycobacterial vaccines need not differ in their response to immunization with an isolated nonviable immunizing fraction. These results suggest that the CF_1 strain of mouse may respond to an immunizing constituent of whole bacilli to which the other mouse strain, the Strong A, does not respond.

On the other hand, mycobacterial strains used as vaccines may behave differently following injection into the two strains of mice. If the attenuated H37Ra and BCG-4 cultures multiply extensively in the organs of the CF_1 mice, as they are known not to do in the organs of Strong A mice,[5] the observed differences in capacity to develop immunity might be explained upon a quantitative rather than a qualitative basis.

Regardless of the mechanism involved, the marked difference in capacity to develop immunity to tuberculous infection noted between the strains of mice poses some very practical problems. For experimental work care should be taken to choose a strain known to give a good immune response to the agent being tested.

This great variation in the capacity of different strains of mice to develop immunity to tuberculous infection also may provide an explanation for many of the numerous discrepancies noted in the literature on the immunogenicity for mice of whole-cell vaccine preparations and fractions of tubercle bacilli.

In summary: The capacity to develop immunity of four different strains of mice has been tested following the injection of BCG vaccine, viable attenuated H37Ra cells, and the immunogenic red fraction obtained from mechanically disrupted H37Ra cells. The capacity of the strains of mice to develop immunity following injection of whole cells varied tremendously. The immune response was greatest in the CF_1 strain, appreciably less in the Strong A strain, even less in the Rockland "all-purpose" strain and very poor in the Rockland "specific pathogen-free" strain. However, although the immune response of mice of the CF_1 strain when vaccinated with viable attenuated mycobacterial cells was very much greater than that of Strong A mice, the immune response of the CF_1 mice was slightly less than that of the Strong A strain to the immunogenic red fraction. The importance of these findings to the problem of the isolation and characterization of immunogenic substances of mycobacteria and to the nature of the immune response in mice has been discussed.

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CONTROLLED CLINICAL TRIAL OF STREPTOVARICIN—ISONIAZID

Initial reports on streptovaricin[1] in 1957 indicated definite *in vitro* activity against tubercle bacilli.[2] In animals, the drug exhibited only a moderate effect when used alone[3, 4] but showed more promise when combined with isoniazid.[4] Experience in man was limited to the use of streptovaricin alone, and in the treatment of 11 patients it was reported to have a clinically detectable antituberculous effect.[4]

At the time when these preliminary results were reported, the United States Public Health Service was conducting a tuberculosis therapy trial on the effectiveness of thirty-two-week regimens of various combinations of isoniazid, streptomycin, PAS, and pyrazinamide. As no serious toxicity was attributed to the new drug, a pilot study on its use with isoniazid was incorporated into the trial already in progress. Investigators[5] in ten of the twenty-five participating hospitals agreed to treat a limited number of patients with this regimen for comparison with those treated with isoniazid—PAS. The procedures used in the conduct of the trial and in the analysis of the results have been described in another report on the trial earlier in this issue.[6]

Any patient at least fifteen years of age with bacteriologic and roentgenographic evidence of pulmonary tuberculosis, and with no more than two weeks of previous chemotherapy, was eligible for the trial. Daily doses of the drugs were 4 to 6 mg. of isoniazid per kg., 3 gm. of streptovaricin (in one dose), and the equivalent of 10 to 12 gm. of acid PAS. Clinical, bacteriologic, and roentgenographic examinations of each patient were made at four-week intervals during treatment.

By June, 1957, 79 patients receiving isoniazid—streptovaricin and 251 patients receiving isoniazid—PAS were under observation. Although the patients receiving isoniazid—PAS were admitted to the trial over a period of a year and those on isoniazid—streptovaricin entered the trial only during the final four months, the characteristics of the two groups of patients resulting from the centrally controlled random assignment of regimens were quite similar, as shown in table 1. Nearly 31 per cent of the patients on each regimen did not finish thirty-two weeks of treatment of assigned therapy for reasons unrelated to their disease or chemotherapy.

Toxicity: Sixteen patients receiving isoniazid—PAS developed toxic reactions severe enough to require a change in the assigned regimen. Two patients (0.8 per cent) could not tolerate isoniazid, 13 (5.2 per cent) were intolerant of PAS, and one was apparently unable to take either drug.

Impurities in one lot of streptovaricin were apparently responsible for the gastrointestinal reactions to streptovaricin which occurred in 6 patients during May, 1957. Toxic reactions in animals were also observed by the manufacturer with this particular lot.[7] No toxic reactions were observed with other lots.

Therapeutic effect: A comparison of the therapeutic effect of the two regimens takes into consideration patients who died of tuberculosis during treatment and those whose regimens were changed because of critical worsening, as well as those who completed the assigned thirty-two-week course. Five patients (6.3 per cent) who received isoniazid—streptovaricin and 2 patients (0.8 per cent) receiving isoniazid—PAS became critically worse during treatment. Two deaths from tuberculosis on each regimen produced a mortality rate of 2.5 per cent for streptovaricin and 0.8 per cent for PAS.

The analysis of roentgenographic change is based on 158 patients who received isoniazid—PAS and on 49 patients who received isoniazid—streptovaricin. The per cent of patients with substantial (marked or moderate) roentgenographic improvement during thirty-two weeks of treatment is shown in figure 1.

The proportion of patients showing substantial

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[1] Generously provided as Dalacin by the Upjohn Company.

[2] SIMINOFF, P., *et al.*: Am. Rev. Tuberc., 1957, *75*, 576.

[3] RHULAND, L. E., *et al* : Am. Rev. Tuberc., 1957, *75*, 588.

[4] MCCUNE, R. M., *et al.*: Am. Rev. Tuberc., 1957, *75*, 659.

[5] Drs. R. J. Davies, B. L. Brock, L. C. Manni, and A. G. Lewis, Florida State sanatoriums at Orlando, Tallahassee and Tampa, Florida; Dr. R. F. Corpe, Battey State Hospital, Rome, Georgia; Dr. L. H. Berard, Municipal Tuberculosis Sanitarium, Chicago, Illinois; Dr. P. T. Chapman, Herman Kiefer Hospital, Detroit, Michigan; Dr. W. J. Steininger, William H. Maybury Sanatorium, Northville, Michigan; Dr. S. Cohen, Glen Lake Sanatorium, Oak Terrace, Minnesota; Dr. H. G. Curtis, Sunny Acres Hospital, Cleveland, Ohio; and Dr. T. F. Sheehy, Firland Sanatorium, Seattle, Washington.

[6] UNITED STATES PUBLIC HEALTH SERVICE TUBERCULOSIS THERAPY TRIALS: Am. Rev. Resp. Dis., 1959, *80*, 627.

[7] MORIARTY, A. J.: Personal communication.

TABLE 1

INITIAL CHARACTERISTICS OF 79 PATIENTS RECEIVING ISONIAZID—STREPTOVARICIN AND 251 PATIENTS RECEIVING ISONIAZID—PAS

Characteristics	Regimen	
	Isoniazid—PAS	Isoniazid—Streptovaricin
Number of patients	251	79
	Per Cent	
Race and sex		
Male white	44.6	55.7
Male nonwhite	17.6	13.9
Female white	19.1	17.7
Female nonwhite	18.7	12.7
Age (in years)		
Less than 35	30.4	29.1
35–54	46.8	53.2
55 and older	22.8	17.7
Stage of disease		
Minimal	15.6	13.9
Moderate	41.4	41.8
Far advanced	43.0	44.3
Cavitation		
None	41.1	38.0
Single	17.1	21.5
Multiple	41.8	40.5

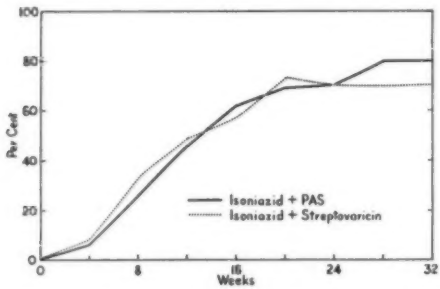

FIG. 1. Percentage of patients showing substantial roentgenographic improvement during thirty-two weeks of treatment with isoniazid—PAS or isoniazed—streptovaricin.

improvement increased steadily and equally on both regimens through twenty weeks of treatment. Patients who received PAS continued to improve while patients on streptovaricin made no further progress. At the end of thirty-two weeks, 70.5 per cent of the patients on the streptovaricin regimen showed substantial roentgenographic improvement as compared with 80 per cent of the patients on the PAS regimen.

Bacteriologic change during treatment among patients with cultures initially positive for tubercle bacilli is shown in figure 2. Reversal of infectiousness among the 46 patients receiving isoniazid—streptovaricin was not as rapid as among the 123 patients on isoniazid—PAS. After the sixteenth week, practically no further change was observed among patients receiving isoniazid—streptovaricin. At the end of thirty-two weeks, 33 per cent of the patients receiving strep-

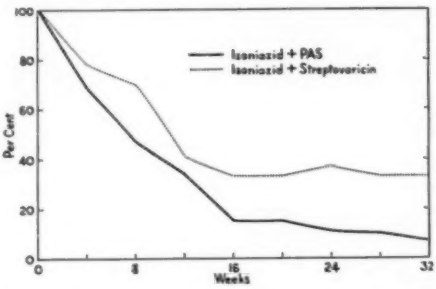

FIG. 2. Percentage of patients with cultures positive for tubercle bacilli during thirty-two weeks of treatment with isoniazid—PAS or isoniazid—streptovaricin.

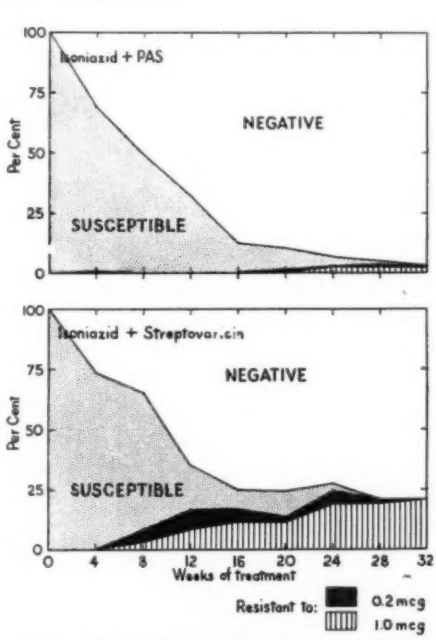

FIG. 3. Results of isoniazid-susceptibility tests during thirty-two weeks of treatment with isoniazid—PAS or isoniazid—streptovaricin.

tovaricin were still excreting tubercle bacilli in contrast to 7 per cent of the patients on PAS. The streptovaricin regimen's deficiencies in reversing infectiousness were statistically significant throughout much of the treatment period.

Drug susceptibility: All cultures positive for tubercle bacilli were tested at the Trudeau Laboratory for susceptibility to all of the drugs used in the trial. As shown in figure 3, resistance to isoniazid appeared much more frequently when isoniazid was combined with streptovaricin than when combined with PAS. Resistance to at least 0.2 γ of isoniazid began to appear after four weeks of treatment with isoniazid—streptovaricin. After twenty-eight weeks of treatment, 21.6 per cent of the patients on this regimen (all those still excreting tubercle bacilli) had organisms resistant to at least 0.2 γ of isoniazid, compared with only 2.8 per cent of the patients receiving isoniazid—PAS.

Streptovaricin-susceptibility tests were performed for all patients receiving streptovaricin and for a small random sample of patients who were receiving other antimicrobial combinations. The emergence of streptovaricin-resistant organisms did not appear to be a significant factor in the drug's lack of therapeutic effect; growth in 10 γ of streptovaricin was slight and infrequent, and occurred no more often among patients receiving streptovaricin than among those treated with other drugs.

No resistance to PAS appeared, except for occasional slight growth in the 1.0 γ concentration.

In summary: A pilot trial of the therapeutic efficacy of streptovaricin in combination with isoniazid was undertaken in ten hospitals participating in the United States Public Health Service Tuberculosis Therapy Trials, with isoniazid—PAS as a control regimen. The results indicate that 3 gm. of streptovaricin daily were inferior to PAS as a companion drug for isoniazid, especially as measured by bacteriologic change. Both the bacteriologic and roentgenographic results obtained with streptovaricin combined with isoniazid are similar to the results observed by this group and by other workers with isoniazid alone. The inability of streptovaricin to delay or reduce the emergence of isoniazid-resistant bacilli may be related to the regimen's ineffectiveness after sixteen weeks.

UNITED STATES PUBLIC HEALTH SERVICE
TUBERCULOSIS THERAPY TRIALS
Tuberculosis Program
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August 12, 1959

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FAILURE TO DEVELOP SARCOIDOSIS AFTER THE ORAL INGESTION OF PINE POLLEN

To the Editor of the American Review of Tuberculosis and Pulmonary Diseases:

Several large epidemiologic studies have shown interesting geographic variations in the incidence of sarcoidosis.[1-4] Cummings and his associates have proposed an explanation which is based upon a relationship with pine trees.[3-5] They have called attention to acid-fast properties of the pine pollen and to certain similarities between substances found in the pollen and in tubercle bacilli. Additional epidemiologic studies are under way which are designed to give further information on whether or not pine trees contain an etiologic factor.

Direct experiments with pine pollen would seem to have many advantages over epidemiologic studies. In order to determine whether or not such studies in humans would be safe, I undertook a study using myself as a test subject. Oral ingestion of the pollen[6] was decided upon because of the convenience and because the macroscopic size of the particles would make it highly unlikely that they would ever penetrate into the deeper areas of the lung. Any such particles which are inhaled would probably be carried back to the glottis, along with mucus, and would be swallowed.

A total of 720 mg. of loblolly pine pollen was prepared in gelatin capsules and ingested in thirty doses between March 7 and May 9, 1959. The only noticeable effect of the pollen was a mild irritation of the bowel with a tendency toward loose stools. To date there has been no roentgenographic or clinical evidence of sarcoidosis. A skin test, using an antigen prepared from loblolly pollen, has remained negative. The strength of the tuberculin reaction did not change.

This experiment does not prove that pine pollen cannot cause sarcoidosis, but it does indicate that direct experiments with humans appears to be safe. Definitive information would probably be obtained by doing studies with patients with sarcoidosis who are in a latent stage of their disease.

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June 23, 1959

[1] MICHAEL, M., JR., *et al.*: Am. Rev. Tuberc., 1950, *62*, 403.

[2] GENTRY, J. T., *et al.*: J. Clin. Invest., 1955, *34*, 1839.

[3] CUMMINGS, M. M., *et al.*: Postgrad. Med., 1956, *19*, 437.

[4] CUMMINGS, M. M., *et al.*: Ann. Int. Med., 1959, *50*, 879.

[5] CUMMINGS, M. M., AND HUDGINS, P. C.: Am. J. M. Sc., 1958, *236*, 311.

[6] The pine pollen and skin test antigen were obtained through the courtesy of Dr. Cummings and Dr. Hudgins.

Books

A TEXTBOOK OF MEDICINE (Tenth Edition). Edited by Russell L. Cecil and Robert F. Loeb. Associate Editors: Alexander B. Gutman, Walsh McDermott, and Harold G. Wolff. Cloth, pp. xxiii + 1665 + lxxxix, W. B. Saunders Company, Philadelphia, Pennsylvania and London, 1959, Price, $16.50.

Few texts have influenced medical practice and understanding as deeply as the *Textbook of Medicine* initiated by Russell L. Cecil thirty-two years ago, and carried on since its eighth edition, in 1951, in editorial conjunction with Robert F. Loeb. The record shows that more than four hundred thousand copies of the first nine editions have been sold. In the tenth edition, appearing, as traditionally, four years after its predecessor, editorial responsibility is shared, as in the previous edition (see *American Review of Tuberculosis and Pulmonary Disease*, 1955, *72*, 695–696), with three Associate Editors, Alexander B. Gutman, Walsh McDermott, and Harold G. Wolff. For these five editors the task of coordinating the large number of individual contributions making up the book must have become a continuous labor. When the text was first issued in 1927, succeeding to the high place of the influential *Principles and Practice of Medicine* of Sir William Osler, it was clear to Cecil that medicine's rapid growth had made it impossible for one author to cover every field. A large number of distinguished physicians were invited to contribute chapters in their own special domains. The practice, which has been continued throughout the years, has given exceptional authority to the text as a whole. One hundred and sixty-four specialists have written sections for the tenth edition. These include four who contributed to the first edition, Cecil himself, and David P. Barr, Franklin M. Hangar and Clyde A. Heatly. Dr. Barr has contributed to all of the ten editions.

Each edition has introduced new names in the list of contributors and new fields for consideration. The turnover is indeed surprisingly large and an indication of the constant labor involved in preparing the text, for each new contributor has developed his field independently, giving a freshness in point of view to the book as a whole. A new note has been added in a foreword by a wise and respected physician, Dana W. Atchley, for whom thousands of patients and colleagues over the years have had affectionate regard. His introduction emphasizes that thorough understanding of disease in the absence of effective communication between doctor and patient is inadequate for the best practice of medicine. In a scholarly book of this sort it is not overly sentimental for such a physician to point out the great importance of "warmth and compassion" in meeting each patient's problems.

The book is too large to permit comprehensive review in a limited space. It seems most practical to orient a review for the particular audience to which it is addressed. Readers of the *American Review of Respiratory Diseases* will have special interest in the sections on viral diseases, tuberculosis and other bacterial infections with a special predilection for the respiratory system, and the wide variety of diseases of the lungs and bronchi of less specific or neoplastic character. Tuberculosis is covered at generous length by J. Burns Amberson, who has added a much needed section on diseases due to "atypical" acid-fast bacilli. The name of John Barnwell, who contributed sections on pleural disease in previous editions, is missed with regret, but these sections have been taken over creditably by William H. Stead. As in past editions, empyema is discussed by William S. Tillett, whose experience in enzymatic treatment as well as chemotherapeutic procedures is well known. Frank Horsfall, Jr., who brought the attention of the National Tuberculosis Association to the enormous significance of viral diseases in this year's J. Burns Amberson Lecture, has written many of the sections in this field, including that on influenza. Pneumococcal pneumonia is treated by W. Barry Wood, Jr., and pneumonias due to viral and other agents by Harry M. Rose. Lowell A. Rantz has covered the streptococcal infections, except for rheumatic fever, which is discussed, as in the previous edition, by Maclyn McCarty. The numerous mycoses, the majority of which may involve the respiratory tract, are covered in detail by David T. Smith. Sarcoidosis has been treated by a newcomer in the list of contributors, Charles A. LeMaistre, with much fresh material on its pulmonary manifestations. Dickinson W. Richards, who is as well known in the field of cardiac disease as in that of respiratory illness, has written the sections on pulmonary function, bronchitis, pulmonary fibrosis, and emphysema. Bronchiectasis, as previously, is discussed by Carl Muschenheim. Cancer of the lung is treated by Herbert C. Maier and the pneumoconioses by Ronald V. Christie.

These names speak for themselves. Their owners have distinguished themselves in investigation and teaching in the fields concerned. They have brought their subjects up to date. Their

discussions are objective, concise and practical. It is regrettable, however, that, large as the book is, most of the individual discussions have to be so short. One would like to see more discussion of staphylococcal infections in hospitals, the relation of tobacco to cancer of the lung, the degree of success of the Salk vaccine, and the highly significant genetic factors in certain diseases of metabolism. All of these are mentioned, but the discussions are of necessity, but still disappointingly, brief.

Special attention should be called, however, to a new and valuable chapter on steroid physiology and metabolism by Joseph W. Jailer, and the growing appreciation, shown in many sections, of the importance of inherent enzyme deficiencies as a cause of manifestations of illness. New material includes information on adenoviral and ECHO viruses, high altitude sickness, radiation injury, agammaglobulinemia, electrolyte deficiencies, and electric shock in the treatment of psychiatric disorder.

The publishers, the W. B. Saunders Company, have kept up with a difficult task in the control of size. The book is large and heavy, but it is sturdy and well printed. The index of eighty-nine pages is highly effective. Mrs. Ann Tourtellot is given credit for proofreading and editorial work; the task was a huge one and well done. Altogether the volume is in the high tradition established years ago. It is sure to strengthen the practice of medicine.

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VETERANS ADMINISTRATION HOSPITALS NUMBER, THE MEDICAL CLINICS OF NORTH AMERICA, January, 1959. The Major Pulmonary Diseases. Benjamin B. Wells, M.D., and Marc J. Musser, M.D., Consulting Editors. W. B. Saunders Company, Philadelphia and London.

The Medical Clinics of North America as a rule seem to serve a basic teaching rather than an informative function in that these symposiums are appropriate for audiences other than those with a special interest in the field under discussion. The volume under review follows this pattern, and recommends itself more to the general physician than to specialists in respiratory illnesses.

A broad range of clinical chest topics is covered in twenty-one chapters. None of these is either exhaustive or analytical. Without exception, however, they are soundly oriented, well-documented, and nicely phrased essays presenting the core of the subject. Controversial material has been avoided and there is little with which a reviewer can take issue.

Several of the chapters should be singled out: "The Specific Diagnosis and Treatment of the Pneumonias" by Wendell H. Hall deals with a large subject with simplicity and clarity. Oscar Auerbach in "The Natural History of the Tuberculous Pulmonary Lesion" offers a lucid and pleasant lecture which I am sure would have been treasured by a medical school class. Patrick B. Storey and Richard F. Kieffer, Jr., under the difficult title of "Trends in the Management of Pulmonary Tuberculosis," have managed a simple and unbiased quick trip through a field in which obfuscations and prejudices abound. The bibliographies have all been carefully selected and are of themselves quite valuable.

The excellence of the Veterans Administration hospitals in pulmonary diseases is always in evidence; this volume is a quite impressive testimony to an extraordinary depth in the field, as well as to a commendable level of style and medical literacy. Although of limited value to the specialist, it would be of definite interest to the general physician, and most of all would be a welcome and most informative guide to the medical student.

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LONG-TERM ILLNESS. MANAGEMENT OF THE CHRONICALLY ILL PATIENT. Edited by Michael G. Wohl, M.D. Pp. 748, W. B. Saunders Company, Philadelphia, 1958. Price, $17.00.

Long-Term Illness fulfills an important educational need in many respects. There is a brief preface which states the purpose and the usefulness of this treatise. The title may be sophisticated, but the message is very much down-to-earth, and emphasizes the change in attitudes and aims which is necessary before approaching the problems of chronic illness.

In the opinion of the reviewer, this is a book with a mission. There is a feeling abroad that current medical knowledge and technical facilities are not being utilized to the maximum in the care of the chronically ill. Some impetus and a new orientation is required to bring these several million people and their unique problems into proper focus. Psychologically, the average practitioner is tuned to the demands of the acutely ill. It is emotionally more satisfying to cure than to assuage. It is more gratifying to maintain or restore the physiologic integrity of a body. Conversely, the effort to salvage tissue remnants or to prolong the function of a feeble organ can be a

frustrating experience. But, needless to say, the chronically ill are deserving of a more equitable share of medicine's resources. This is so not only because these people are our patients, but all too frequently because they are our parents or children.

The text is divided into two sections. The first ninety pages deal with general principles of care. This section might more aptly be titled "Total Patient Care." It includes a wealth of practical information for contending with the situations which are inherent in chronicity. The second section deals with the treatment of specific chronic diseases; and the twenty-nine eminently qualified contributors cover the field quite thoroughly. The emphasis again is on specific methods in patient care. But a little more is added. The patient with a long-term illness frequently has an assortment or an accumulation of possibly unrelated diseases. The physician and others participating in the total care program will be alerted to many features of care which do not directly apply to the primary disease. Chronic diseases are by their nature progressive diseases, and preventative measures are appropriate at every stage of the disease process. This is a concept not often defined in other medical texts.

In brief, the two sections may be summarized with the admonition that the patient with a long-term illness requires total patient care; and the means for achieving this are presented.

A few more random comments are in order to note that the index is adequate, and the table of contents breaks down the material into categories which make for easy reference. Each chapter is subdivided by numerous bold-faced titles; and the pages are printed in two columns. These are welcome attributes in the format of the book which certainly facilitate reading. On the whole it is an excellently edited, and well-published text which deserves wide attention.

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BOOKS RECEIVED

AUTOMATIC VENTILATION OF THE LUNGS. By William W. Mushin, L. Rendell-Baker, and Peter W. Thompson. Pp. 349, Charles C Thomas, Springfield, Illinois, 1959. Price, $10.50.

AN INTRODUCTION TO MEDICAL GENETICS. By J. A. Fraser Roberts. Pp. 263, Oxford University Press, London, New York, 1959. Price, $7.00.

RESPIRATORY PHYSIOLOGY AND ITS CLINICAL APPLICATION. By John H. Knowles. Pp. 256, Harvard University Press, Cambridge, Massachusetts, 1959. Price, $5.25.

TUMORS OF THE LUNGS AND MEDIASTINUM. By B. M. Fried. Pp. 467, Lea and Febiger, Philadelphia, 1958. Price, $13.50.

LA TUBERCULOSE DES HOUILLEURS. By Jacques Prignot. Pp. 327, Editions Arscia S. A., Brussels, 1959.

THE ECOLOGY OF HUMAN DISEASE. By Jacques M. May. Pp. 327, MD Publications, Inc., New York, 1959. Price, $7.50.

PREVENTIVE MEDICINE IN WORLD WAR II. VOLUME IV: COMMUNICABLE DISEASES. Editor in Chief, John Boyd Coates, Jr. Pp. 544, Office of the Surgeon General, Dept. of the Army, Washington, D. C., 1958.

American Trudeau Society

Medical Section of the National Tuberculosis Association

The following dates and locations of the next five annual meetings of the National Tuberculosis Association and the American Trudeau Society have been selected and approved:

1960—Los Angeles, California—May 15–20
1961—Cincinnati, Ohio—May 21–26
1962—Miami Beach, Florida—May 20–25
1963—Denver, Colorado—May 12–17
1964—New York, New York—May 24–29

NEW YORK TB AND HEALTH ASSOCIATION OFFERS GRANTS IN RESEARCH AND MEDICAL EDUCATION

Fellowships and grants amounting to $100,000 currently are being offered by the New York TB and Health Association under its recently expanded research program. The most distinguished is the James Alexander Miller Fellowship, which carries a stipend of $7,000 to $10,000. Other fellowships carrying a smaller stipend are offered and priority is given to New York City area applicants. Grants for the support of work of established researchers whose studies have a potential relationship to the problems of tuberculosis are also offered. To qualify for such grants, the work must be conducted in medicine, sociology, epidemiology, or the basic sciences, and the project must be conducted in an approved university, hospital, or other institution in the New York City area.

Applications for fellowships must be made by November 15; applications for grants-in-aid are due at least six months prior to the date the contemplated project is scheduled to start. Application forms may be obtained by writing to the New York TB and Health Association, 260 Park Avenue South, New York 10, New York.

FORTY-NINTH STATE ORGANIZES A TRUDEAU SOCIETY

The Alaska Trudeau Society was organized in Anchorage on August 18, 1959. American Trudeau Society members residing in Alaska and other physicians interested in respiratory diseases attended this organizational meeting. The officers elected were: President, Dr. Francis J. Phillips, Anchorage; Vice President, Dr. Ernest W. Gentles, Seward; Secretary-Treasurer, Dr. Jose L. Silva, Anchorage. In addition to the officers, the following were elected to the executive committee: Dr. Jack W. Gibson, Juneau; Dr. John I. Weston, Fairbanks; and Dr. David L. Sparling, Mt. Edgecumbe.

NEW SOCIETY ORGANIZED IN ECUADOR

La Sociedad Ecuatoriana de Especialistas del Torax, with a broad field of interest in respiratory diseases, has recently been organized. It will publish an official journal to be known as *Revista Ecuatoriana de Enfermedades del Torax*. The first officers of the new Society are as follows: President, Dr. Jorge Higgins; Vice-President, Dr. Amadeo Moreira; Secretary, Dr. Ernesto Briones; Treasurer, Dr. Enrique Ortega; Secretary of Scientific Sessions, Dr. Carlos von Buchwald.